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FURTHER STUDIES ON "MAGNETOSENSITIVE BACTERIA"

Beginning in 1958, it was observed that some bacteria, comprising various morphotypes, displayed the surprising behavior of always migrating toward the geomagnetic North Pole, even when the orientation of a sample on a microscope slide was changed by rotating the stage of the microscope. At first, there was some doubt, but after facilitating a rather rough plan of research, we were forced to accept what had been randomly observed. Despite a justifiable skepticism, a series of scientific investigations were organized in our Institute. In 1961 we were able to account for the results obtained with an internal report in the Institute itself.

MATERIALS AND METHODS

Samples of fresh water were collected at different depths by means of long pipettes, at times extracted from glass tubing heated with a Bunsen burner and stretched at one end, or using test-tubes immersed in water with maximum caution in order to not mix up the layers of water and keeping the bottom end plugged until the established level was reached. This technique demonstrated that the useful water levels were between one and seven centimeters from the surface of the pool of water, depending on the depth of the sample of water taken (lake shore, wetland, ponds and permanent bogs, ditches and so forth) and at any rate until reaching the boundary between the oxygenated and deoxygenated water. It was possible to deduce the boundary from the color of the sediments. The oxidized layers were rusty, whereas the deoxygenated ones were dark brown, grayish or blackish. Based on these empiric observations, it was possible to conclude that the bacteria with magnetic sensitivity preferred an a very low oxygen, if not anoxic, environment. This agreed with their behavior in the geomagnetic field that induced them to head towards the bottom of the natural basin in which they had lived, meaning towards the sediments, and at the same time away from high oxygen that could be damaging for them (fig. 1).

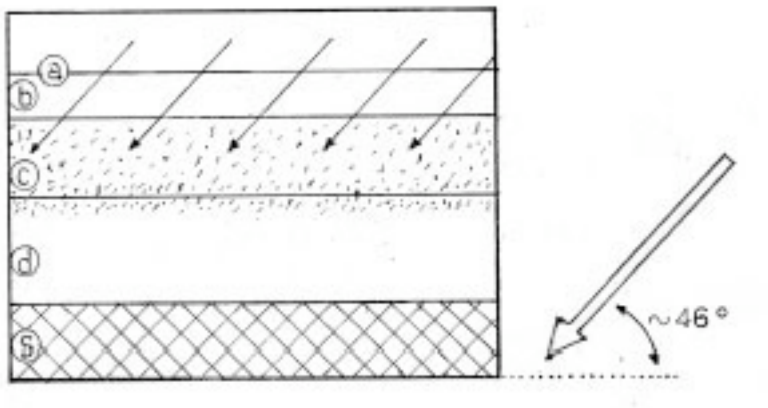


Fig. 1

a: surface of the pool of water
b: oxygenated layer
c: transitional layer from the oxygenated one to the hypo-oxygenated one
d: layer of hypo-oxygenated to anoxic
s: sediment
⟶ orientation of the movement of the Magnetosensitive Bacteria and the geomagnetic field

(For the small mass and the relatively limited velocity of the bodies being examined (the bacteria) the factitious force of the "Coriolis Effect" was not taken into account)

The samples collected included water and the solid component that is the sediment, the latter comprising a third and at times a fourth of the total volume. The samples were examined in part within three up to eighteen hours after the collection and in part during the following days; these were placed in *bèute*, some filled 4/5 up, or were left in the collection recipients closed with hydrophilic cotton or with sterile rubber caps. They were kept at room temperature in the cabinets or also in a thermostat at 20° C to 23° C for periods of one or more weeks. In this situation the containers were not hermetically sealed modest volumes of sterile water were added at times. It was important that they were maintained in dim light. For samples of larger quantity, containers (of small capacity and sterilized pots) were used closing them with their lids and in these cases the bobbins (which will be discussed ahead) were built on tubes of isolating material, of the type used in building or in road work, of adequate diameter, despite the problem of the quantity of wire necessary for the solenoid, in part overcome with the technique suggested by Helmholtz. Also used were Petri dishes, emptied, sterilized of a given circumference in which were introduced the samples being examined to expose to the magnetic fields.

The selection of the Magnetosensitive Bacteria was carried out using bar magnets or electromagnets: between the first 25-70 minutes a copious quantity of bacteria would accumulate relative to geomagnetic North as confirmed by the needle of a compass placed parallel to the magnet at a proper distance.

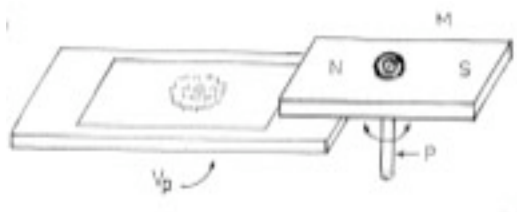
The material containing the bacteria in question, isolated this way, was pipetted into *bèute* or sterile vials containing fresh water coming from ditches or from ponds and processed through centrifugation to separate the solid parts; what remained on the surface was filtered and sterilized. The microorganisms collected were washed in a similar manner.

In another approach, enrichment cultures were made by leaving the material by itself in the collection containers and maintained in a thermostat at a temperature between 20° C and 23° C. A transparent door in the thermostat allowed some faint light but not direct sunlight to reach the sample. During the summer, the thermostat was often not used, and the collected material was maintained in a secluded place with dim light.

With the goal of incubating the sediment and the samples containing the bacteria in total rest mode, test tubes of large diameter were used for direct macroscopic observation. These were placed in test tube holders provided with a mirror inclined at 45°,which allowed the sample to be viewed, particularly the bottom with the solid mass, that is, the anoxic zone, or the oxygenated zone. Between observation periods the mirror was covered by thin, black, removable cardboard to shield excess light impinging on the sample.

The separation of the Magnetosensitive Bacteria in the cultures was initially obtained with simple equipment: a) a bar magnet with a brass or hardwood hinge at the center to enable rotation (fig. 2 a-b) so that the orientation and polarity of the magnetic field at the sample could be reversed; b) an electromagnet made with a coil wrapped on a core of pure iron and connected to one of the various transformers, in which the dc current could be changed mechanically or by relay.

a)



b)

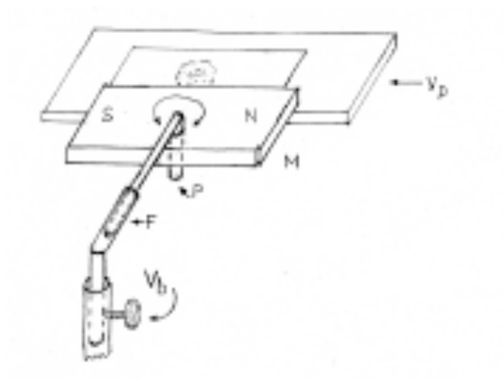
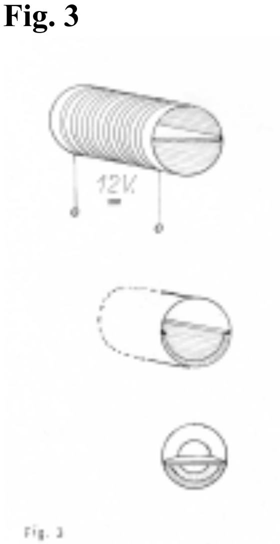


Fig. 2 (a/b)
Use of small magnetic bars for studying the Magnetosensitive Bacteria:
M: magnetic bar
N/S: magnet polarity (reversed)
Vp: slide with the microscopic preparation
P: brass or hardwood pivot on which the magnet bar is mounted
F: friction for the horizontal movement
Vb: Lock-Screws of the vertical movement

A more refined method of separation (and of enrichment) of the Magnetosensitive Bacteria consisted in winding glass-lined copper thread, or copper covered with another insulating material, on a Bakelite tube with a 26 millimeter internal diameter, so that a glass microscope slide could just fit inside. As an alternative, the support for the slide could consist of half a tube of Bakelite of about 25 millimeters external diameter, cut longitudinally with a small saw and set with putty inside the outer tube that was wrapped with the copper windings.

Fig. 3



The coil was built with reconciled coils and was connected to a reostate transformer of the type used for miniature electric trains with a continuous, well-filtered exit current, (of 1 to 12 V./ 1.5 A.), that allowed reversal of polarity in order to obtain the reversal of the magnetic flux: the north-south orientation was verified with a compass placed at adequate distance (fig. 3 and 4). In the effort to define the most useful voltage and the most useful amperage as well as to observe the behavior of the bacteria in magnetic fields of different characteristics, electromagnets were supplied with up to 850 V./1 A. and the behavior of the Magnetosensitive Bacteria was evaluated in different field strengths with the results described in the previous report (*).

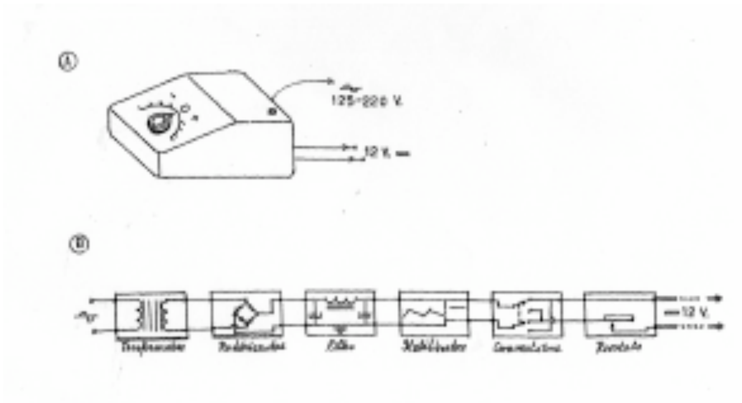


Fig. 4

A) Drawing of a model of power pack for miniature electric trains

B) Block outline of an electric power pack with outrigger: output of 12 Volts at 1.5 Amp.

In the end, the low voltages mentioned above were chosen to preserve the safety of the experimenter, having established that at those low voltages all the experimental results were overlapping and potentially equivalent. The Bakelite tube was replaced with a glass tube in order to observe the migration of the bacteria.

In another method, a Helmholtz coil arrangement was made of thin wire and flattened with a hammer or with another appropriate tool, wrapped either on a tube or on the microscope slide (fig. 5 a-b).

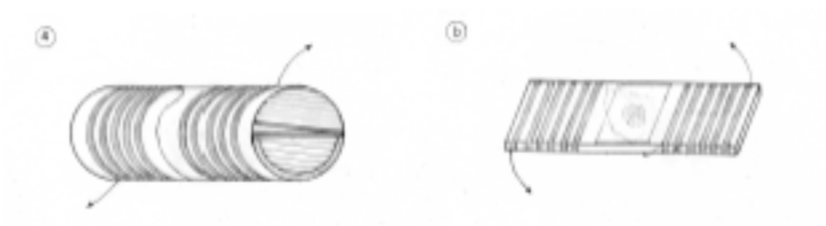


Fig. 5 – *descriptive drawings of Hemlholtz Bobbins involved in the experiments.*

The pair of coils conceived by Helmholtz generated a uniform magnetic field as if they were a single wrapping of length equal to twenty times its own diameter and beyond.

Enrichment of the cultures was obtained by duplicating the methodologies described with periodic addition of iron salts or traces of dusty rust ($2[Fe_2O_3 \cdot nH_2O]$) or finely pulverized, friable material. The same procedure was followed in order to restore the magnetosensitivity of the bacteria in the enrichments which were potentially attenuated in soils devoid of metal compounds.

Once the Magnetosensitive Bacteria were separated, we proceeded to cultivating them in small rectangular glass tubs, or in Petri dishes, on semi-solid soil standard for each type of microorganism, to which iron salts were added, or on culture soil prepared without iron salts. The growth of the colonies in semi-solid soil was normal and they accumulated toward the side of the Petri dish, or of the small tub, oriented to geomagnetic North even when the position of the container was rotated or changed. If the soil of the culture was solid, modifying the orientation of the containers didn't show significant variations of the location of the bacterial colonies during the relatively brief times of observation. The examination of the bacteria was not continued past a predefined time period because the results were considered unnecessary for the goals of the research. Nevertheless, it was observed that after a few weeks the colonies of the Magnetosensitive Bacteria had expanded toward the geomagnetic North in some containers of solid soil which had been left alone after the end of the experiment.

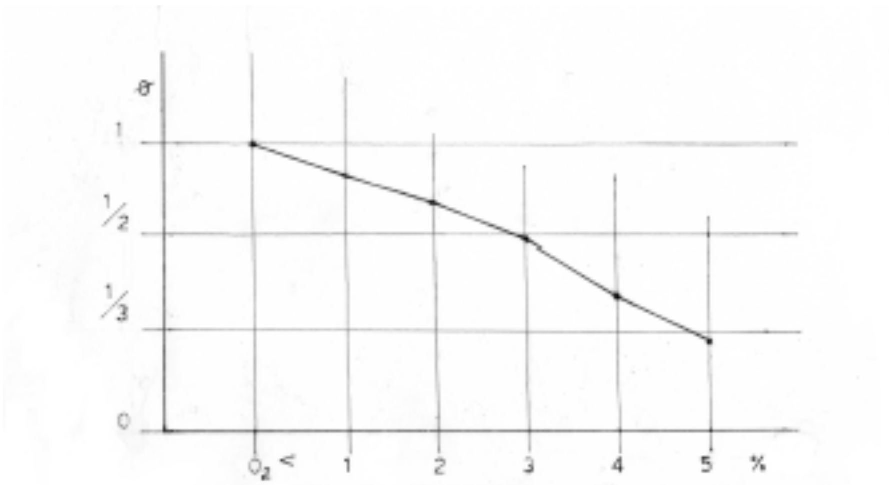
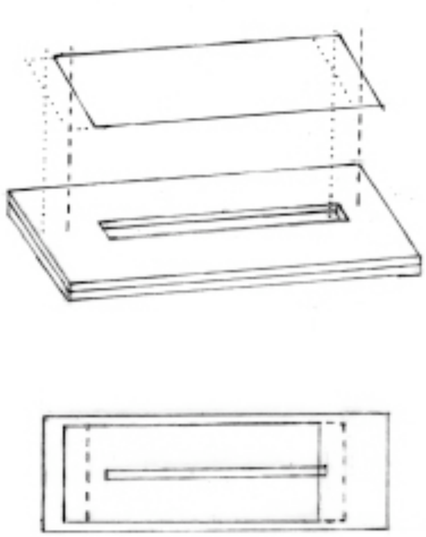


Fig. 6 – Growth curve, expressed in diameter, of the bacterial colonies, in relation (inversed) to the percentage concentration of O2 in the surrounding air: as the percentage of O2 was growing, it was observed the decrease in the development of the bacterial colonies. *The values read are arbitrary, even if they refer to the actual ones; the only goal of the chart is to make the phenomenon visible and of intuitive understanding.*

Some cultures, executed in parallel and prepared in *bèute*, were enriched with oxygen, the others enriched with nitrogen in progressive quantities with the goal to examine the percentage of each gas that would be optimal for the development of the colonies (fig.6)

To reduce the amount of air, and therefore of the oxygen, in the containers, a simple method was used. After heating to a maximum of 28° C, the containers were immediately closed with sterile airtight caps and sealed with paraffin. Another way was to connect the *bèute* to a pump that would extract some of the air, and replacing it with gaseous nitrogen. Ultimately, a calculated amount of gaseous nitrogen was simply introduced to the bottom of the containers in proximity to the sediments without preventively extracting the air, with no major nonconformity in the results.

Fig. 7 – Drawing of the micro-rooms used



A further experiment included the use of two overlapped slides. A window was cut out in the upper slide to create a rectangular micro-room, 3x36 mm, and the upper slide was then attached to the lower slide with water-resistant putty. Finally, the assembly was sterilized (fig. 7).
A thin layer of sterile water was put in the micro-room and at the center a drop of material containing the Magnetosensitive Bacteria. Then the microroom was covered with a 24x50 mm coverslip, leaving only a slit at the end to allow the introduction of oxygen or nitrogen at the glass-water interface. Gas was introduced through a piece of equipment like the ones used to administer oxygen to people with asthma, and the end of the coverslip was slid over the end of the micro-room and coated with glycerin in order to contain the introduced gas. The spring clips located on the stage of the microscope were used to increase the cohesion of the coverslip on the slide. Initially the slide was not placed in a local magnetic field, that is, it was left in its natural environment. In a second experiment, a local magnetic field was created by means of a magnet or electromagnet. In the first case, the Magnetosensitive Bacteria introduced in the micro-room of the slide migrated with barely noticeable speed away from the oxygen bubble. In the second case, the bacteria migrated at higher speed, which varied depending on the orientation of the magnetic poles of the magnet in relation to the natural geomagnetic field. In a further test, only gaseous nitrogen was introduced into the same slit and a slow movement of the bacteria toward the nitrogen bubble was observed, however not very clearly. Therefore this was not very convincing. But if the gas was then substituted with oxygen, the Magnetosensitive Bacteria returned to the initial position, even going past it. The experiment was repeated using a 24x32 millimeter coverslip so that a slit would remain at each of the two extremities of the micro-room. A drop of bacterial culture of a single magnetosensitive species, cocci, bacilli or even spirilli, was placed at the center of the micro-room. While the cocci appeared to move in a single direction, bacilli and spirilli could eventually be seen at both extremities of the micro-room. This suggests that they are capable of moving in both directions along the magnetic field in search of an environment adequate for their physiology.

Nonmagnetic microorganisms, in the same samples that did not orient or migrate along the magnetic field toward geomagnetic North were also cultivated for comparison tests. Cultivation occurred in culture soils (devoid of iron) identical to the ones used for the Magnetosensitive Bacteria. Even with the naked eye, differences between the magnetosensitive and non-magnetosensitive colonies of bacteria were detected through the coloration of the colonies, which were grayish-black for the magnetosensitive organisms, while they were a delicate yellow-pink for the non-magnetosensitive ones. In each case, when deemed useful or necessary, a solution of NaOH or of citric acid was used to readjust the pH of the culture soil toward neutrality.

The chamber for the study of thick layers of biological material also comprised two overlapping slides. A 10-15 millimeter diameter circular hole was cut in the top slide, and then it was sealed to the bottom slide with putty or Canadian Balm, and dried at room temperature. The chamber was covered with a coverslip used as a support of the material being examined. A second chamber consisted in binding washers of adequate diameter (not metal, but of the type used by plumbers or found on the seals of small bottles) to a slide, and closed with a coverslip, with the goal of studying the biological material in a hanging drop, particularly with dark field illumination. Washers were also obtained by slicing rubber tubes filled with paraffin, which was then removed. Various magnifications were used for the observation (*).

Dark field microscopy was achieved by raising the microscope condenser to the slide, opening the diaphragm to the maximum, and inserting a small opaque disk in the center. The condenser bulb had to be high intensity but not so high as to damage the preparation under examination. Simple coloration with Methylene blue showed that the Magnetosensitive Bacteria were Gram-negative.

CONCLUSION

In a previous contribution (*), that must be considered a “precautionary note”, the possibility was raised that in nature there existed bacteria that orient themselves with respect to the geomagnetic field. The present study has verified this finding by various methodologies and has demonstrated that this behavior is linked to a kind of biological compass placed inside the bacterial cell body. The phenomenon is found in various morphotypes, including spirilli, cocci, and bacilli.

Applying magnetic fields of alternating polarity, either by spinning a magnetic bar equipped with a central pivot, or changing the direction of the current in an electromagnet, it was possible to make the magnetosensitive bacteria change their direction of movement. That is why it is likely that in the southern hemisphere of our Planet, similar bacteria migrate toward the southward. Through use of the the micro-room method, it was verified that the bacilli and the spirilli, but not the cocci, migrated toward the two extremities, as long as oxygen was not introduced through the slits. When oxygen was introduced at the extremities, the bacteria returned toward the center of the micro-room, where the percentage of oxygen was, without a doubt, less.

When bacteria killed with heat were subjected to a magnetic field, they would spin around themselves without showing any translation. It is presumed that the hypothesized magnetic compass is tied inside the body of the cell, and is comprised of iron compounds. It is further presumed that the bacterium would make use of the chemical modality of orientation, perhaps with the goal of removing itself from concentrations of chemical elements that are damaging. The recurrent presence of iron in the oxygenated environment is striking, which suggested the use of rust in the enrichment cultures. Therefore, magnetosensitivity is a means to point toward a profitable niche for survival and multiplication.

It would be satisfying to examine the behavior of the bacteria at the Earth’s equator and at the poles, to compare/check the scientific truth of the hypothesis formulated here on the basis of the research results. An investigation of the magnetosensitive bacteria strains with an electronic microscope would likely illuminate many of the advanced assumptions.

DISCUSSION

It doesn't seem superfluous, with the goal of best understanding the phenomena investigated here, to mention certain aspects of magnetism. Each magnetic object has lines of force that run symmetrically between the two opposite poles, but at the galactic scale the symmetry changes due to the intervention of other phenomena such as the solar wind. It is known that the planets of the solar system, excluding Venus and perhaps Mercury, are magnets and particularly planet Earth, which is our center of interest, is a magnet because of shifts of charges in the internal layers. According to a recent hypothesis, protons and electrons moving with different modality and speed would generate a rotating current that produces the geomagnetic field, which, added to the force of gravity, would have a great importance either for the formation of matter or for its evolution.

The geomagnetic field is detectable over the entire planet and its flux lines point from the South Pole toward the North Pole. However, the geographic poles do not exactly coincide with the magnetic poles, which have reversed during certain Geological Periods. That is. the North Pole has become the South Pole and vice versa. This effect has occurred on average every half million of years in the long run. To this day, the cause of these reversal periods is an object of conjecture, as well as of short duration (named "Magnetic Events"), documented by paleontological finds.

Some materials, particularly iron, if introduced in a magnetic field, become magnetized because their atoms behave like coils on which current travels, aligning themselves in the same direction. The relation between the intensity of the magnetic field present in the material in an eternal magnetic field and in its absence is called "magnetic permeability" and the level of magnetization is expressed by the size of "μ" characteristic of each material. For iron, the magnetic permeability is large and can reach values much higher, even if not constant, because it increases in direct proportion to the external magnetic field, until the maximum value due to the total alignment of the atoms. Its relative permeability is equal to 5000 μ, but can reach much higher levels in particular alloys. The magnetization can endure in permanent magnets or for brief temporal periods.

The relation between electricity and magnetism is experimentally emphasized by wrapping a solid coil conductor (solenoid) usually, but not necessarily, of cylindrical shape in which is introduced a microscope slide or a piece of white cardboard dusted with iron powder, which, when the electric current passes through the solenoid arranges itself along the flux lines of the magnetic field, allowing visualization in this manner. The intensity of the magnetic field is in direct relation with the number of coils, their thickness, contiguity and the diameter of the solenoid.

As far as what was said above, it appears licit to wonder if the movement of the Magnetosensitive Bacteria generates energy directly proportional to the extent of the variation of the flux and inversely proportional to the time of variation considered in relation with elements, mineral substances or biological entities, that can be functionally identified with the coils of a solenoid, or at any rate, with structures capable of transforming the work (movement of the microorganism) into electrical forces (or electromotive forces) depending on the induction principle. Obviously this cannot be assessed for a single bacterium, but could become a calculable entity for a conspicuous plurality of bacterial cells.

This aspect seems important for the ecological and biological organization of our planet, particularly if we think of microbes in terms of cosmic objects inserted in a uninterrupted chain that links the inorganic kingdom (isolated minerals or compounds that the bacteria assimilate in order to conjugate simple molecules into more complex ones, or to separate the complex into elementary molecules or yet to release them in the environment at the end of their life cycle, as they are valuable to the plant and animal kingdoms.

It isn't clear if the Magnetosensitive Bacteria migrate toward the North Pole (and presumably toward the South Pole in the southern hemisphere) exclusively for magnetic orientation or to search for sites with low oxygen content or anoxia, obeying the criterion of optimal concentration of oxygen, as if they had the capacity to assay and evaluate the relative quantity of oxygen in the environment. It seems necessary to ask whether the bacteria use their magnetosensitivity and their sensitivity to chemical concentrations alone to seek their preferred environment, and if their "sinking" toward the silt is governed by only these two stimuli, or if they mask other demands and other meanings that at the present state of our understanding escape us. The fact is that the magnetosensitivity and the oxygen concentration in the environment appear to be the decisive factors that together address the search for the optimal niche for the life, the growth and the propagation of the Magnetosensitive Bacteria, which for this purpose migrate most likely using, as already said but doesn't hurt repeating, simultaneously magnetic and chemical procedures. It must be equally conclusively determined if and how they assemble the existing material in a confined environment, in this case a laboratory receptacle, for the construction of the internal "compass" only when the oxygen concentration does not exceed a certain percentage. In reality, it was noticed that in enrichment cultures of semisolid soils maintained in confined circumstances and containing a constant quantity of iron compounds, as the oxygen percentage decreased, the bacteria slowly gained back the magnetization they had lost as if they had been cultivated in soils deprived of magnetizable metal compounds.

In nature, it could be possible to verify a virtuous circle for which, in a determined site, some factors, including in particular, the iron compounds released by the dead bacteria, can form ambient areas in which the surviving or growing microorganisms are capable to find the material either to grow or to build their own compass. At the end, it seems logical to surmise that the manufacturing of the hypothesized internal compass of the Magnetosensitive Bacteria is a biological operation perfectly organized and controlled in every aspect and at each step, that is, in the composition, the production, the optimal quantity, or in the allocation inside the bacterial cell. If it is conditioned by the presence of minerals and specifically iron, deposits of such substances should be found, presumably in the sediments, where the bacterial body has plummeted to dissolution. In fact, the Magnetosensitive Bacteria are detected in amounts of water with overlapping layers, although not rigorously distinct, of different chemical composition such that the upper layer is more oxidized and the lower layers are gradually less oxidized down to the anoxic layer. The bacteria are found just above the latter layer, though it is not hard to find some in the anoxic zone as well. The layers with more bacteria are those with relatively greater, but still low, concentrations of oxygen. These layers contain suspended solids of pinkish-brown color. It remains to determine whether the Magnetosensitive Bacteria are born magnetized or are only predisposed to become magnetized and they get their compass through the influence of various nutritional and environmental factors existing at the site where the progenitors were located by migration before giving life to the new generation.

This last hypothesis entails the possibility that other bacterial strains can be magnetized and others still – beyond these detected, studied and made known – can acquire the magnetosensitivity even only temporarily for purposes or for reasons that, if the expressed suppositions were confirmed, will have to be investigated.

(*) *Bellini, S. – About a particular behavior of fresh water bacteria, (1963), Institute of Microbiology, University of Pavia*

[A special thank you to the master glazier of Ditta Spelta di Pavia for his proved patience, unity in the ability in the production of the micro-rooms]

SUMMARY

In this further investigation the Author expose the materials and methods of study on Magnetosensitive Bacteria and argue the results. A large discussion winds up the study.

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