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Stéphane Tirard
François Viète Center for Epistemology and History of Science and Technology, University of Nantes
stephane.tirard@univ-nantes.fr

Abstract

This paper proposes a historical survey of the research conducted over three centuries on microscopic animals capable of entering into a latent form of life. Concentrating on the 17th and 18th centuries, it starts with the first observations and experiments on these animals made by naturalists during the 18th century. It then focuses on the 19th century, exploring French biologists' interest in this topic, focusing on the views of Paul Doyère and Claude Bernard. It also analyzes how, at the beginning of the 20th century, Paul Becquerel used his works on latent life to reject the panspermia theory. Finally, it shows how since the middle of the 20th century, artificial methods of cryoconservation were developed in parallel with studies on natural biological phenomena, leading to discussions on the nature of life itself. The conclusion claims that a comparison between natural phenomena and methods of artificial conservation can help us form a global conception of latent life, thereby advancing the debate over the nature of life.

Keywords : Cryptobiosis, cryobiosis, cryoconservation, latent life, definition of life

Introduction

The property of latent life is shared by many species, and is observed in nature as well as being artificially triggered in laboratories, where very low temperatures are used for the conservation of cells. This paper proposes a historical survey of the research conducted on microscopic animals capable of entering into latent life, which have actively aroused the interest of naturalists and biologists for over three centuries (Keilin 1958; Tirard 2010). Here, we will trace scientific work and ideas on this issue across two centuries, showing how latent life came to be explained in terms of the organization of the living matter in an animal (the preservation of a specific form as much as of the matter itself) and how the phenomenon has been linked to both evolutionary discourses and reflections concerning the nature of life.

After a presentation of the earliest observations and experiments concerning the phenomenon of latency dating from the 18th century, this paper focuses on the contributions of French biologists to this topic, in particular the views of two very influential scientists, Paul Doyère and Claude Bernard. The positions adopted by these two men had a considerable influence on European biology at the beginning of the 20th century. Doyère was the first to express his thoughts on the importance of the preservation of the organization of matter during a period of latency. Later, Bernard claimed that latent life was a form of life that was useful for studying the most fundamental phenomena in living matter. The article will also show how, at the beginning of the 20th century, Paul Becquerel used his work on latent life to refute the panspermia theory. The first half of the twentieth century saw an increase in detailed studies of latent life as a natural phenomenon with the various mechanisms involved the subject of separate investigations. Starting in the middle of the 20th century, scientists started to develop artificial methods of cryoconservation. The overall goal of this paper, which we make explicit in the conclusion, is to show that the comparison between the natural phenomena associated with latency and the artificial laboratory-based methods of conservation can help us formulate a global conception of the nature of latent life and so of life in general.

The first observations of the phenomenon of latency in animals

In 1702, Antoni van Leeuwenhoek (1702a, b) described the microscopic animals or animalcules "found in sediments in the gutters of the roofs of houses", which he named *rotifers* (Ratcliff 2000). Under certain conditions these animals could desiccate and stay inert for months, re-establishing their living activity upon rehydration. At the time, the property was termed *resurrection*, a relatively common word at the time that did not necessarily bear any religious connotations. During the 18th century, several naturalists, like Maurice Roffredi, Felix Fontana, Henry Baker, John Needham and Lazzaro Spallanzani, made a great many observations and experiments on rotifers, tardigrades and nematodes (called eels at the time).
Spallanzani's experimental methodology was particularly interesting (1769), especially since they contributed to his argument against spontaneous generation as the mechanism leading to the appearance of life on Earth. Firstly, he tried to identify which animalcules possessed this property of latency, demonstrating that the majority of species could not come back to life after significant loss of water and that resurrection was possible only in rotifers, tardigrades and eels. Secondly, he made detailed observations of these different animals and studied how many cycles of drying out and re-hydration (i.e. the transformation to a latent form of life) were possible for these animals under different conditions. He also studied their resistance to various extreme conditions of temperature.
Finally, he focused on the nature of the state of these organisms when they had been dried out. Were these animals dead or alive? This question, which, if we consider the experimental methods he used, Spallanzani addressed empirically, was evidently very philosophical. One could argue that his experimental results could have allowed Spallanzani to conclude the existence of an inert state that was not death. However, in the last lines of his paper, he claimed that this inert state did not exist and that a very fine thread of life persisted in these animals even when they were desiccated.

Spallanzani's opinion has to be understood in the context of his wider work on the question of generation, where he was interested not just in spontaneous generations but in procreation more generally. In a very lively debate with Needham on the topic of spontaneous generation, the central point they discussed was the formation of eels in wheat disease. Needham claimed that these animalcules arose through spontaneous generation, while Spallanzani was convinced that they came from a previous generation, following a stage of latent life. In other words, there was a "fine thread of life" that continued unbroken from one generation of eels to the next. There was an analogous opposition between the two naturalists on the issue of procreation. Needham was an epigenist and Spallanzani believed in the pre-existence of germs, i.e. in the continuity of the organization of living beings from generation to generation. Therefore, we can see that across these three different, but related domains of research Spallanzani was convinced of the continuity of life. He clearly opposed the idea that each individual organism could be the result of its own, *sui generis* auto-organizational capacities.
Another more speculative conclusion we can draw is that, for Spallanzani, latent life represented the minimal possible level of life, allowing him a means to prove the continuity of life in other situations by analogy.

Michel Doyère: the conservation of the organization of matter

During the 19th century, the investigation of latent life mainly focused on identifying the state of matter in desiccated animals. In France there was a lot of interest in this topic throughout the century, as evidenced by the following examples.
In 1842 Louis Michel Doyère published his thesis dedicated to targigrades in Paris (Doyère 1842), where he extended the experiments of Spallanzani and of the French naturalist René Dutrochet (1837) with precision, providing a very interesting interpretation of latent life at the scale of the matter composing the animals. In the conclusion to his thesis, Doyère argued that there was no interruption of life and that desiccated animals possessed "life *in potentia*", and with the return of water to their bodies they returned to a state of "life *in actu*". According to him, the important issue was the preservation of organic organization during the period of desiccation. In other words, during a period of latent life, metabolism stopped but the organization of the matter of the organisms remained unchanged.
Latent life continued to be actively discussed in France right up until the 1850s, setting the scene for the high-profile debate between Felix Pouchet and Louis Pasteur over the question of spontaneous generation. As we will explain in more detail in what follows, latent life played an important but discrete role in the exchanges between Pouchet and Pasteur. Pouchet held a position quite different from that of Doyère, claiming that when dried out the animals were not still living but rather that the resulting destruction of their organic material killed them outright. For Pouchet, what was termed resurrection was in fact a case of spontaneous generation in which the dead matter was reorganized into a living animal.

In 1859, the French *Society of Biology* organized a debate on this topic. Over the course of 42 sessions a commission auditioned members of the two opposing schools of thought and then Paul Broca (1860), the president, wrote a report. Broca's conclusion was clearly in favour of Doyère, considering that the inert state preserved the structure of the matter that constituted these organisms. This episode illustrates the immense challenge faced by biologists when trying to explore this phenomenon. The difficulty lay in the technical challenges of obtaining evidence concerning not only the state of dryness of the animal tissues but also the absence of metabolic activity in an organism.

There is a clear link between the Society of Biology's debate on latency and the debate on spontaneous generation that took place several months later. This new debate, launched by the French Academy of Sciences, opposed Félix Pouchet as a supporter of spontaneous generation and Louis Pasteur, its leading detractor . During the 1850s, Pouchet actively defended his ideas on heterogeny, a putative phenomenon of spontaneous generation based on the transformation of dead matter (Pouchet 1859). The story of how Pasteur's numerous experiments helped him to argue that spontaneous generations did not exist has been amply commented in the history of science. What is less well known is how closely connected these two issues – latent life and spontaneous generation – were at the time. Two aspects of the same broad issue of the organization of living matter, the opposing sides in both these debates embody two opposing conceptions of life. According to Doyère and Pasteur, there was continuity in living phenomena, based on the conservation of the organization of this matter. According to Pouchet, this organization did not persist, yet could re-emerge spontaneously (from once living but now inert matter) following the destruction of an organism.

Claude Bernard: conceiving a state without any metabolism

Latent life continued to be a subject of interest to biologists, with the French physiologist Claude Bernard, for example, assigning a central place to the phenomenon in the conception of life that he presented in his *Lectures on the Phenomena of Life Common to Animals and*

Plants. (Bernard 1966) According to the French philosopher Georges Canguilhem, this was a book of "biological philosophy" in which Bernard integrated a presentation of physiological data and concepts with his own philosophical reflections on life.

A short presentation of the first chapters or lectures will help to illustrate how central latent life was to this major work of physiology from the second half of the 19th century. The first lecture, which had no title, covers both the definition of life and the goals of physiology. In this lecture, Bernard had this to say about life:
"I consider that two orders of phenomena are necessarily in a living being:
1° The phenomena of *vital creation* or *organizing synthesis*;
2. The phenomena of death or *organic destruction*."
This led to his famous definition of life linking it with death via a complementary pair of claims: "Life is creation" and "Life is death". This definition exercised an extraordinary influence in academic spheres, providing a reference for a number of discussions on the nature of life.
In the second lecture, entitled *The Three Forms of Life*, Bernard presents life in terms of a constitutive balance between complementary forces: "a close harmonious relationship between exterior conditions and the pre-established constitution of the organism. It is not by a struggle against the cosmic conditions that the organism develops and maintains its existence; on the contrary, it is by adaptation and blending with these cosmic forces" (Bernard 1966: 67)
Then he goes on to describe the three forms of life
"1. Latent life: "life is not manifest";
2. oscillating life: "variable manifestations depending on the external environment (the case of the tree)";
3. constant life: "life with free manifestations which are independent of the external environment" (Bernard 1966: 67)
According to this point of view, drying out and the passage to latency is a means for the organism to conserve its life in the form of latent life, when exposed to extreme environmental conditions. For the organisms capable of latent life, this is the condition for survival. This form of life concerns not only animals (rotifers, tardigrades, nematodes…) but also plants in the form of seeds.
Furthermore, Bernard pushed his analysis of latent life to the level of the cells and more particularly of the protoplasm, in which, according to him, the process of life continued. He considered the mechanisms by which the state of latent life could be maintained and those deployed for the return to manifest life. Here, it is useful to refer to the definition of life given in the first lecture. Indeed, it is the external conditions that influence if not determine the two orders of phenomena observed in living beings: creation and organic destruction. According to Bernard, desiccation is the first condition for the passage of life from its manifest to its latent form. Dessication has the immediate effect of halting organic destruction, which, along with creation, is one of the terms from his definition of life. He added that, at the chemical level, the ferments become dry and inert, thereby blocking the chemical phenomena of vital destruction. In arguing this point, Bernard drew on the work of Chevreul, a French chemist who had studied the capacity of certain tissues or organic bodies – including tendons, fibrous tissues, albuminoid substances and ferments – to dry out and rehydrate. In a second phase, creation is also halted. In this way, life is "suspended", in a state of "chemical indifference" or neutrality. (Bernard 1966: 98) Finally, the return to manifest life begins with the restoration of organic destruction, thanks to the re-activation of substances accumulated before the passage into the latent phase of life, and the restoration of destruction is followed by the restoration of creation.

In the following lectures Bernard focused on the importance of the protoplasm, arguing that the protoplasm was the site of the fundamental living phenomenon of simultaneous organization and destruction. He presented latent life as the perfect case for studying this precise equilibrium.

Latent life and the origin of life

Paul Becquerel's work at the beginning of the 20th century signals a transition with the previous period. Throughout his career, this French biologist specialized in the study of latent life. He prepared his PhD under the direction of the French botanist Gaston Bonnier, who had a great interest in the topic of latent life, notably with respect to seeds. (Van Tieghem & Bonnier 1880) During the first decade of the 20th century, Becquerel looked at whether or not a very low metabolic activity existed during the state of latent life, conducting numerous experiments aimed at elucidating the phenomenon of latent life in seeds. According to him, in the absence of water and oxygen, under almost zero pressure and in temperatures close to absolute zero the protoplasm becomes rigid, as hard and as inert as a stone. The colloidal state had, according to Becquerel, totally disappeared, leading him to claim that his experiments had proved an end to all life in the case of certain seeds. (Becquerel 1906a; b; 1907, 1909, 1910a). He also denied any law of continuity of life, arguing the opposite, that an interruption of life was possible (1910).

Moreover, like other French botanists, Becquerel was very interested in the question of the origin of life. It is, indeed, interesting to note that a tightknit group of French botanists had widely divergent opinions on evolution and origins of life. (Tirard 2013) For instance, Philippe Van Tieghem (1891) was Darwinian and panspermist, while two of his students, who would become rather influential figures in French academia, Jules Costantin (1923) and Gaston Bonnier (1907), were Neolamarckian. The former believed in evolutionary abiogenesis (Kamminga 1988), a progressive evolution of matter that was the source of life, and the latter was panspermist. Becquerel decided to test this hypothesis of panspermism, which was well known thanks to its defense by Thomson in 1871 (Thomson 1871; Tirard 2006) and its later detailed development by Svante Arrhenius. (Arrhenius 1903) In this essentially chemical theory, during their formation, at a point when they were sufficiently cool, planets would be receptive to the arrival of microscopic germs of life that were present at all times everywhere in space. Therefore, Becquerel performed some experiments aimed at clarifying the issue of the resistance of seeds and spores to the physical conditions likely to be encountered in space. He showed that they could not survive radiation with ultra-violet rays, concluding from this that panspermia was impossible. (Becquerel 1910b) It is difficult to evaluate the influence of these results on the decline of the panspermia theory, but what is clear is that the theory was soon abandoned with the issue of the origin of life coming to be dominated by evolutionary abiogenosis hypotheses.

Natural cryptobiosis and artificial conservation techniques

The second half of the 20th century was particularly active when it comes to latent life. On the one hand, natural phenomena continued to be studied and, on the other hand, a series of practical processes were developed to artificially induce the state.

In 1958, Daniel Keilin proposed the name cryptobiosis to characterize an extremely low level and yet reversible metabolic state. (Keilin 1959). The diversity of states of *cryptobiosis* was studies for a wide range of species and several categories were differentiated with respect to the level of resistance to different physical and chemical factors.

Anhydrobiosis is the ability to dry out, historically demonstrated for rotifers, tardigrades and nematodes in the 18th century, as we have seen. In the early 1980s, it was established that some tardigrades species are common in polar regions, and the term *cryobiosis* is now used for this extreme form of resistance to freezing, characterized by the metabolism coming to a halt. *Osmobiosis* qualifies the ability to support variations in salinity, and concerns several species of tardigrades, and the capacity to endure a lack of oxygen, which probably appeared early in marine environments, is called *anoxybiosis*.

The cellular and molecular mechanisms of this form of conservation have been subjected to intensive studies. (Wharton 2002) Nowadays, biologists understand the dehydration phase, which characterizes latent life, as a metabolic process that protects cell structures from the processes of death: irreversible damage to membranes and macromolecules, release of free radicals, racemization of molecules, etc. The conservation mechanisms depend on the presence of cryoprotective molecules, in particular Trehalose, which is naturally produced in animals in latent life, plays a major protective role throughout the process. Therefore, it is possible to speak of a specific state of biological organization associated with latent life. (Clegg 2001)
Biologists have suggested biological explanations for the elements of different life cycles, including periods of latent life, which are taken to correspond to a form of adaptation to seasonal cycles as well as to the protection against various extreme conditions, such as those of extreme cold or drought. Moreover, biologists have proposed an evolutionary hypothesis for the acquisition of the capacity for cryptobiosis (Jönsonn and Jämero 2003), notably based on studies of the sea arthrotardigrades, the most primitive tardigrades, which do not possess this capacity (Kinchin 1994, 75).

On the practical side, several scientific fields, and notably certain domains of medicine have come to use freezing techniques to preserve organic specimens. This technique is commonly used for conserving cells in laboratories and for conserving plant tissues in the context of collections of varieties or species. These techniques are important in the field of artificial procreation techniques involving humans and other animals, where laboratories freeze spermatozoa, embryos and, more recently, oocytes for use at a later date.
Historically, since the end of the 1940s, this field of artificial procreation has benefited from research done on Bovinae, in which techniques for freezing embryos and spermatozoa were first developed. Poldge and colleagues greatly advanced the development of the nascent field of cryobiology by showing that glycerol could be used to protect many species' sperm when cooled to very low temperatures (Poldge 1949). These techniques ushered in a very active period of empirical research, and attempts were made to develop appropriate protocols, notably using new cryoprotectants. In the late 1970s, Willadsen developed an effective method by which he was able to obtain a survival rate which was only slightly lower than that of fresh embryos for implantation in cattle. In 1976, the first cattle exports arrived in New Zealand in frozen form, a cost-effective export method given the small size of embryos, even if they needed to be kept in freezers. Subsequently the same techniques were applied to human reproduction. The *in vitro* fertilization and embryo transfer conducted by Robert Edwards and Patrick Steptoe led to the emblematic birth of Louise Brown (the first 'test-tube baby') on 25 July 1978 and the first human embryos were successfully frozen in 1983. Today, the cryoconservation of spermatozoa, embryos and, more recently, oocytes have become commonplace. One could argue that these practices correspond to the artificial introduction of stages of latent life into the life cycle of human beings.

Conclusion: What are the perspectives for a global concept of cryptobiosis?

If we consider latent life as a whole, there are, on the one hand, the natural processes with their long history of scientific research, and on the other hand, the methods of cryoconservation which are more recent only really becoming practicable since after the Second World War. Methodologically speaking, the former research is characterized by observations and experimentation on the resistance of organisms and associated research into the cellular and molecular mechanisms involved in latency. The latter techniques, sometimes grouped together as cryobiology, are more closely tied into effective methods of conservation. Would it be possible to consider that these two fields form a single unified domain? This unification is difficult, for at less two reasons. Firstly, as shown above, the two fields are based on different methodological approaches with different aims and theoretical perspectives. The first field studies natural phenomena, the second artificially induces an analogous state. Secondly, a great diversity of disciplines, with their own intellectual and institutional boundaries, are integrated into each of the two fields, including but not limited to zoology, biology, microbiology, and medical sciences, which makes any conceptual synthesis very difficult.

However, one could argue that this unity already exists on the conceptual level, and it seems that a global theorization could, indeed, be developed. For instance, it would be interesting to consider the significance of the introduction of a period of artificial latent life in the biological cycle of humans, when spermatozoa, embryos or oocytes are frozen. In this state, are they in cryobiosis, as defined by Keilin? It seems possible to respond in the affirmative, since these cells show no sign of activity. The cell metabolism is not measurable, and it seems to be completely stopped, but this state is reversible. Moreover, this state, imposed by physicians or their technicians to protect spermatozoa, embryos or oocytes is analogous to natural situations. It is interesting to underline the fact that the molecular principles of protecting cells are closely comparable in the natural and artificial cases. This sort of linkage could be the first step in the emergence of a unified concept of cryptobiosis.

We can finish by posing a central question: would reaching such a unified concept help in the ongoing debates concerning the nature of life? (Tirard, Morange, Lazcano 2010) An affirmative response to this question also seems plausible. Cryptobiosis, whether natural or artificial, provides very interesting data on the limits of living systems and helps us to clarify certain conceptual issues. The comparison between the inert and the active state allows us to analyze the limits of the complex relationships between molecular and cellular structures and metabolic processes. Moreover, attempting to define the cryptobiotic state is undoubtedly a useful heuristic exercise, as it requires if not a definition at least a reflection on the nature of life.

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