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The origin of symbiogenesis: An annotated English translation of Mereschkowky's 1910 paper on the theory of two plasma lineages

Klaus V. Kowallik, William F. Martin *

Institute for Molecular Evolution, Heinrich-Heine-University of Düsseldorf, Düsseldorf, Germany

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This paper is dedicated to Miklós Müller, biochemist and historian of science, on the occasion of his 90th birthday.

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ABSTRACT

In 1910, the Russian biologist Konstantin Sergejewitch Mereschkowsky (Константин Сергеевич МереЖковский, in standard transliterations also written as Konstantin Sergeevič Merežkovskij and Konstantin Sergeevich Merezhkovsky) published a notable synthesis of observations and inferences concerning the origin of life and the origin of nucleated cells. His theory was based on physiology and leaned heavily upon the premise that thermophilic autotrophs were ancient. The ancestors of plants and animals were inferred as ancestrally mesophilic anucleate heterotrophs (Monera) that became complex and diverse through endosymbiosis. He placed a phylogenetic root in the tree of life among anaerobic autotrophic bacteria that lack chlorophyll. His higher level classification of all microbes and macrobes in the living world was based upon the presence or absence of past endosymbiotic events. The paper's primary aim was to demonstrate that all life forms descend from two fundamentally distinct organismal lineages, called mykoplasma and amoeboplasma, whose very nature was so different that, in his view, they could only have arisen independently of one another and at different times during Earth history. The mykoplasma arose at a time when the young Earth was still hot, it later gave rise to cyanobacteria, which in turn gave rise to plastids. The product of the second origin of life, the amoeboplasma, arose after the Earth had cooled and autotrophs had generated substrates for heterotrophic growth. Lineage diversification of that second plasma brought forth, via serial endosymbioses, animals (one symbiosis) and then plants (two symbioses, the second being the plastid). The paper was published in German, rendering it inaccessible to many interested scholars. Here we translate the 1910 paper in full and briefly provide some context.

Background. The primary split among living things that Mereschkowsky (1910) suggested corresponds to an almost clean divide of what we now call prokaryotes (mykoplasma) from what we now call eukaryotes (amoeboplasma), names that would not enter the literature until 1925 and would not come into common use until the 1960s (Katscher, 2004). Because Mereschkowsky grouped the fungi together with the bacteria, he missed the prokaryote eukaryote dichotomy we now recognize. The fungi have always been problematic: "*Fungorum ordo in opprobrium artis etiamnum Chaos est, nescientibus Botanicis in his, quid Species, quid Varietas sit.*" (The order of the fungi is still a disgrace to the discipline [of classification], as botanists have yet to ascertain what is a species and what is a variety. Linnaeus, 1751).

The traits Mereschkowsky used for classification are physiological, emergent from a set of dichotomies that distinguish different kinds of cells with regard to:

- Oxygen respiration: anaerobes ancient, aerobes derived;
- Temperature: thermophiles ancient, mesophiles derived;
- Nitrogen requirement: assimilation of inorganic N ancient, organic N derived;
- Cytoplasmic movement: non-streaming cytoplasm ancient, streaming cytoplasm derived;
- Chemical composition: high P ancient, low P derived; high N ancient, low N derived;
- Tolerance of cytotoxins and harsh environments: extremophiles ancient, others derived;
- CO_2 assimilation: autotrophs ancient, heterotrophs derived; plus a few other traits (high Fe ancient, low Fe derived), cell wall (nitrogenous cell wall ancient, cellulose cell wall or no cell wall derived), ability to form true tissues (absence ancient, presence derived), and chromatin (presence ancient, absence derived).

The last criterion, absence of chromatin being derived, seems odd, but for Mereschkowsky the amoeboplasma was the "pure" cytosol of

* Corresponding author.
E-mail addresses: klaus.kowallik@hhu.de (K.V. Kowallik), bill@hhu.de (W.F. Martin).

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plant and animal cells, cytosol without organelles. Plant and animal cytosol lacks demonstrable chromatin. Chromatin in the nucleus was, in Mereschkowsky's view, the result of bacterial intruders, endosymbionts that "… *assembled in the cell's center and finally surrounding themselves by a membrane, thereby formed the cell nucleus. The cell nucleus opened up completely new possibilities with regard to the further evolution of the Monera. Without this symbiosis the anuclear Monera would have been condemned for ever to remain the same lowly life form that they originally were.*" (from the full translation in this paper). In that passage, it sounds like Mereschkowsky was suggesting that symbiosis was the key hurdle to eukaryotic complexity. Yes, that is exactly what he was saying.

Mereschkowsky had to invoke all manner of convergence to explain the origin of traits among the fungi that conflicted with their grouping with bacteria. We have flagged some of those passages in the text. For example, he saw respiration in fungi as analogous, not homologous, hence convergent to that in plants and animals. He interpreted the nucleus of fungi as convergent to that in plants and animals, not as the product of symbiosis, and the cytoplasmic streaming of fungi as analogous, not as homologous, to cytoplasmic streaming in plants. He attributed the diversity of form among plants and animals to the diversity of their enzymes, which in his view were synthesized by the nucleus because of the exceptional protein synthetic ability of the bacterial endosymbionts from which it stemmed. That concept, namely that increased protein synthesis in nucleated cells was a consequence of the first endosymbiotic event in eukaryote evolution, is now a widely recognized component of endosymbiotic theory, although it took 100 years to resurface (Lane and Martin, 2010). The first demonstrable endosymbiosis in eukaryote history involved mitochondria, organelles that Mereschkowsky ignored, not bacteria that congregate in the center of the cell to surround themselves by a membrane and thereby form a nucleus.

One wonders why Mereschkowsky did not adhere more closely to Occams's razor by placing fungi among the amoeboplasma so as to define eukaryotes in a modern sense and avoid complicated explanations involving convergence for fungal respiration and nuclei. The text provides clear clues as to why he grouped fungi and bacteria together as the mykoid kingdom. In the passages on tolerance to harsh conditions, he emphasizes the robustness of fungi towards extreme environments as a strong character linking them to bacteria. On chemical composition, the presence of N in the cell wall is also interpreted as a strong character linking fungi to bacteria. But one character in particular stands out in this regard, namely his reliance upon a small number of papers that reported the growth of fungi in the presence of N_2 as the sole nitrogen source. He viewed the ability to fix N_2 as extremely ancient, just as the ability to fix CO_2 was ancient in his view, and the first organisms he inferred (micrococci) were able to do both without the help of chlorophyll. Today we would call that chemolithoautotrophic origins as it relates to the origin of life (Preiner et al., 2020), an idea that was well ahead of its time. As it pertained to his classification scheme, he had the right interpretation (N_2 fixation is ancient), but the observation was erroneous (diazotrophic fungal growth), leading him to place fungi within the mykoid kingdom, closer to *Clostridia* than to animals. By weighting the tendency of fungi to tolerate extreme conditions and their ability to assimilate inorganic nitrogen sources (erroneously including N_2) more heavily than respiration or the presence of a nucleus, he put them on the wrong side of what became the prokaryote eukaryote divide. Mereschkowsky failed to incorporate anaerobic eukaryotes into his scheme, although he was aware of them, mentioning the anaerobic ciliates, including *Nyctotherus* (Boxma et al., 2005), which possesses hydrogenosomes (Müller, 1993) in footnote 5. Because of fungi, he ultimately named the entire realm of bacteria as mykoids (Greek *mukēs*, fungi) derived from mykoplasma, rather than bacteria derived from bacterioplasma.

Using physiological traits, the 1910 paper fleshes out the foundation for his initial exposé (Mereschkowsky, 1905) of what we today call endosymbiotic theory, or symbiogenesis, to use the original term. In the 1905 paper he made a very strong case for the endosymbiotic origin of plastids. In the 1910 paper he (rightly) considered the plastid pillar of the theory to be so obviously correct that it needed neither further evidence nor argumentation. As such, plastids themselves play only a minor role in the 1910 paper.

In order to better understand the title and the main message of the paper — "*zwei Plasmaarten*" — which translates literally to "two species of plasma", we have to consider the mindset of biologists in 1910. Physics already had the Planck constant and relativity, chemists were already celebrating decades of colorful diazo dyes and the first plastics (Bakelite), while biologists did not have much more than the educated guess as to what was going on in cells. For example, Mereschkowsky cited work by Pflüger in which it was suggested, in some detail, that the CO_2 exhaled during respiration did not derive from ingested food but instead was emitted from the chemical backbone of proteins through a myriad of tiny high temperature explosions. Otto Warburg's work had not yet transformed the field, his first papers appearing in 1905 (Krebs, 1972; Höxtermann, 2007). It would be 1929 before Lohmann discovered ATP (Langen and Hucho, 2008). Given that biologists had no energetic or chemical basis to understand what cells are or how they work, what did Mereschkowsky mean with the term "plasma"? He meant protoplasm.

The concept of protoplasm, *Protoplasma* in the abundant German literature of the 1800s, was omnipresent in the biological sciences in 1910 and roughly as mainstream as it gets. It was still in wide use up until about 1960. Protoplasm is a concept with its own interesting history (Liu, 2017), the term tracing to the Czech and German physiologists Jan E. Purkinje and Hugo von Mohl. It became linked with various concepts, *inter alia* that a special life energy, vital force, or *vis vitalis* is associated with living substance. Strong proponents of that view were called vitalists, their opponents mechanists (Geison, 1969). In the absence of a chemical understanding of the life process within cells, protoplasm represented a special kind or organization of matter that bestows the property of life and distinguishes living from non-living things. Literally it is the first plasm (*protos*, Greek first) and represented a continuous lineage via cell cytoplasm that is the thread of continuity in life across countless generations from origins to the present, and that irreversibly dissolves at death. In his book *The Protoplasmic Theory of Life*, Drysdale (1874, p. 5) described protoplasm like this "… *the elements are in a state of combination not to be called chemical at all in the ordinary sense, but one which is utterly sui generis. That, in fact, no albumin, fibrin, myosin, protagon, or fats exist at all in the living matter, but that the sum of the elements of all these is united into a compound, for which we have no chemical name, and the complex mode in which the atoms are combined we can form no idea; and it is only at the moment of death that those chemical compounds, with which we are familiar, take their origin. […] Vitality is thus a property inherent in each particle of the living matter, and all the parts of a complex organism differ in function, each part has a specific kind of vitality peculiar to itself.*" Such was the nature of protoplasm.

Among other things, the concept of protoplasm conveniently

displaced the burden of understanding how the life process inside the cell actually works into the inaccessible realm of understanding how life arose at its origins. The papers in volume 30 of *Biologisches Centralblatt* in which Mereschkowsky's paper appeared were replete with the term protoplasm. Mereschkowsky used it dozens of times, and we can be certain that different authors meant different things when they used it. As the origin of life was seen in 1910 as a singular event in the primordial phases of Earth's history, the origin of protoplasm and the origin of life were, to many biologists of the time, the same thing. It was not until about 1920 when biological chemists started getting a handle on enzymes that convert small molecular weight compounds during metabolism, such that the notion of protoplasm having special properties fell quietly out of favor.

Mereschkowsky, however, was convinced that he had identified two kinds of (proto)plasm that were so different in nature that they only could have arisen independently from one another, as opposed to one being derived from the other via direct filiation. The consequence of that, in his view, could only mean one thing: life arose twice. He had already mentioned this in the closing passages of his 1905 paper. In the 1910 paper we are given the underlying observations plus the fuller reasoning that led him to that conclusion. According to Mereschkowsky, the first kind of (proto)plasm to arise was robust in nature, corresponded to autotrophic bacteria that had not yet evolved chlorophyll, and appeared shortly after Earth's formation at a time while the Earth was still hot (prokaryotes). The second kind (*Art*) of (proto)plasm arose later, after autotrophs had generated organic substrates to support their heterotrophic lifestyle and was more fragile, less thermophilic and less able to tolerate extremes in its overall nature (eukaryotes). In the final pages of the paper, Mereschkowsky makes that case explicitly, using comparative cytology and physiology in a rationally staged early Earth history context.

In that sense, there is a case to be made for translating the 1910 title as "two origins of life", which is what he argues in the paper, but not what he wrote in the title. Rather the title focusses on two kinds of protoplasm whose differences explained the deepest and most fundamental spilt in the living world, notwithstanding a few corollary convergences among fungi. The two kinds of plasma furthermore retained their ancestral properties even in the wake of ancient symbiotic associations within the same cell. Plastids for example, as the seat of autotrophy in plants, remained recognizable as descendants of cyanobacteria (mykoplasma) living in an amoeboplasma cell. Thus, Mereschkowsky was thinking in terms of two independently arisen plasma *lineages* that united to form complex cells. Moreover, the unification of those *lineages* with persistence of their properties, together with occasional endosymbiont loss, form the basis of life's highest level classification. Any questions as to whether Mereschkowsky was thinking in terms of lineages and lineage diversification are answered by the lone figure at the end of the paper.

For those reasons, we translate *Art* (kind, type, species) in the title "*Theorie der zwei Plasmaarten* …" as lineage, "The theory of two plasma lineages …", because it was not just the fundamental differentness of the plasmas but also a distinctive immiscibility of their properties that persisted despite ancient symbiotic associations, one in the animal lineage and two in the plant lineage. That persistence allowed the endosymbionts and their host to be recognized as independent *lineages* (organellar and cytosolic) even within modern plant and animal cells, as his figure unmistakably depicts. Mereschkowsky could have easily entitled his paper "*zwei Protoplasmaarten*" and it would have been synonymous with the title he selected.

For today's microbiologists, the excitement that extremophiles have always harbored as providing windows into ancient life and origins will seem very familiar in Mereschkowsky's 1910 paper. We have not hyped up any passages, taking every effort to convey the emphasis and level of conviction of the author, also in those passages where he was clearly getting it all wrong. We have, however, cut some of the very long and complicated sentences into two, sometimes three, shorter and simpler sentences.

Many terms in the paper such as Monera, mykoids, amoeboids, infusoria, protoplasm, sarcode and others are no longer in use today. Instead, we are familiar with the terms describing a cell as being either prokaryotic or eukaryotic and cytoplasm an aqueous protein solution (cytoplasm is about 400 mg/ml protein), a product of gene expression, not a kind of matter comprised of molecules that are themselves endowed with special innate properties lacking in other organic material. Some terms that he used have changed meaning over the years, for example Zellmembran (cell membrane) was used to designate the bacterial cell wall up until the 1950s, Mereschkowsky used Zellmembran to designate cell walls in bacteria in some cases. Infusoria could mean several things from ciliates to diverse pond water protists and it is not always clear which meaning he intended, hence we just stuck with infusoria.

Several of Mereschkowsky's ideas were afloat in various manifestations at his time. The concept of the Monera, uptake of organisms from different phyla, incorporation into the host cell, endosymbionts living in subordination to the cytoplasm while being transformed into new organs of the new organism of higher rank, had been mentioned occasionally in the American, German and Russian literature around the turn of the 19th to the 20th century. Famintzyn (1907), for example, was an early proponent for symbiosis as a mechanism generating new forms, in particular lichens. But for perspective, Famintzyn (1907) wrote "The equivalence *[by Mereschkowsky]* of plastids and cyanobacteria is pulled out of thin air, as is the claim by the author (p. 601): 'that plastids are cyanobacteria that invaded the cytoplasm.'" Famintzyn criticized both Mereschkowsky for not knowing the literature and August Weismann for his "strange" *[eigentümliche]* theory of evolution involving a germline.

Mereschkowsky's intuition allowed him to incorporate a fairly vast spectrum of observations into a new theory, the theory of two (proto) plasma lineages. Remarkably, he interpreted all plant and animal cells as still harboring both kinds of plasma in a form that had not undergone hybridization or homogenization of their properties. That reflects the strength of his conviction that the main physiological properties that separate plants from animals reside within plastids, which he saw as irrefutably descended from cyanobacterial endosymbionts. The scientific historical context in which Mereschkowsky found himself, as well as accounts of his troubled personal life are given in Höxtermann (1998), Sapp et al. (2002) and in chapters of the volume by Geus and Höxtermann (2007). The 1910 paper was published during his time of employment at the University of Kazan 1902–1914. Mereschkowsky had politically influential adversaries who drove him out of Kazan in 1914 (Höxtermann, 1998).

The concept of secondary and tertiary endosymbioses with eukaryotic algae as endosymbionts was unknown to Mereschkowsky and only proposed much later by Sarah Gibbs (1978), well after electron microscopy had revealed the number of membranes surrounding the plastids in different groups. Based mainly on pigmentation, Mereschkowsky thought that the plastids of red algae, green algae, brown algae, diatoms

etc., resulted from seven independent symbioses involving different cyanobacterial progenitors. This idea of polyphyletic plastid origins was discussed well into the 1980s, yet the evolutionary hurdle of inventing a protein import machinery favored a single origin (discussed in Cavalier-Smith, 1982). Plastid genomes resolved the issue though, as they left no doubt that the DNA in different plastid lineages was descended from a single successful primary event involving one cyanobacterium as endosymbiont taken up by a heterotroph (Kowallik, 1989). From that symbiosis, the primary plastids of glaucocystophytes, red algae, and green algae emerged, the latter two subsequently giving rise to secondary symbioses among the green and red lineages (Kowallik, 1993). Though he missed secondary symbiosis, Mereschkowsky did point out that lichens are the result of a threefold (*dreifache*) symbiosis.

Endosymbiosis in evolution is, however, a Pandora's box, because once one has accepted the principle that symbiotic associations can give rise to novel organelles (mitochondria and plastids), and taxa at the highest ranks (eukaryotes and algae), what constraints tell us where to stop invoking additional symbiotic events to explain various aspects of cells? That problem has always plagued endosymbiotic theory since its inception. The creative nature of line drawings to represent lipid bilayers was an advance of 1960s electron microscopy. It formed the basis of Lynn Margulis' proposition that eukaryotic flagella arose from symbiotic spirochaetes (Margulis et al., 2006). Line drawings have also been used to suggest a symbiotic origin of peroxisomes and even the endoplasmic reticulum (discussed in Martin, 1999). Line drawings also underlie modern incarnations of Mereschkowsky's 1910 proposal that the nucleus arose from an endosymbiotic intruder within an anucleate host (López-García and Moreira, 2020). In a modern context, that theory (López-García and Moreira, 2020) predicts that the cytosolic ribosomes of eukaryotes should be of bacterial rather than of archaeal origin. But the observations soundly reject that idea. There are no bacterial ribosomes in the eukaryotic cytosol that would betray a spirochaete origin of flagella, and there are no bacterial ribosomes in the eukaryotic cytosol that would betray a δ-proteobacterial host for an archaeal nucleus. The only bacterial ribosomes in eukaryotes are in mitochondria and plastids, those in the eukaryotic cytosol are archaeal, indicating that the host for mitochondria was an archaeon (Martin et al., 2015; Imachi et al., 2020).

In the course of publishing this paper, two readers asked "What about Lynn Margulis and the origin of mitochondria?" We and others have explained in earlier writings that the priority for the symbiotic origin of mitochondria does not go to Margulis (Sagan 1967), nor does it go to Altmann (1890), whose bioblasts were not mitochondria despite many claims to the contrary. Priority might go to Portier (1918) in French, but in our view should probably go to the American cell biologist Ivan Wallin, who had the basic idea so right that he even predicted gene transfer from organelles to the nucleus (Wallin, 1925, 1927). Margulis (Sagan) wrote on the second page of her 1967 paper "… these ideas are not new …", mentioning Mereschkowsky and Wallin but not saying a word about what they had written on symbiogenesis.

Margulis learned about endosymbiotic theory at the University of Wisconsin in her undergraduate genetics class held by Hans Ris, who wrote in 1962: "*With the demonstration of "nucleoplasm" in chloroplasts, the similarity in ultrastructural organization of a chloroplast and a blue-green algal cell becomes indeed striking. Both are enveloped in a double membrane. Both contain the photosynthetic apparatus in membrane systems of similar organization […]. Both contain particles which look like ribosomes in the electron microscope. Whether they are in fact ribosomes remains to be established by isolation and biochemical analysis. Both contain DNA in the form of a nucleoplasm; i.e., areas of low density which contain fibrils about 25 Å thick. We suggest that this similarity in organization is not fortuitous but shows some historical relationship and lends support to the old hypothesis of* Famintzyn (1907) *and* Mereschkowsky (1905) *that chloroplasts originate from endosymbiotic blue-green algae*" (Ris and Plaut, 1962, p. 388). How do we know that she heard about endosymbiosis in that class? We know that because Jonathan Gressel (pers. comm. to WM) at the Weizmann Institute, sat next to Margulis in Hans Ris' genetics class and told us about it. Margulis popularized endosymbiotic theory but did not rediscover it, she was taught it.

In the old days, biologists were taught Occam's razor, that explanations of unknowns are first to be sought in the terms of known quantities. More so than any other evolutionary mechanism, endosymbiotic theory requires restraint. It should only be used in explanatory emergencies, as a last resort when all other evolutionary mechanisms fail, as in the origin of mitochondria and photosynthetic eukaryotes. Endosymbiotic theory also works best when founded in physiology, rather than in line drawings that purport to represent the evolution of thin sections as viewed through the electron microscope. If one asks: What membrane systems in cells might we explain as the result of endosymbioses, many possibilities come to mind: The nucleus? Flagella? Peroxisomes? The ER? The problem with endosymbiosis is that it is so interesting as an evolutionary mechanism that it opens the floodgates to overuse — for each eukaryotic membrane we see, we can just add one more endosymbiosis. But where to stop? When is enough? If we stick to the physiological foundations of endosymbiosis and ask "What physiological or thermodynamic conditions favor symbiotic associations" (Imachi et al., 2020) we obtain welcome constraints on the number of cellular partners that a symbiosis can support. Endosymbiosis should only be invoked when standard evolutionary mechanisms fall short.

What is so special about endosymbiosis? Endosymbiosis creates a unique physical relationship between cells, one within the other, that alters the fate of genes and membrane vesicles that are naturally released by the endosymbiont. The release of genes to the host is the source of the lineage transforming power of symbiosis that generates new taxa at the highest level via cell combination during evolution. Yet symbioses involving prokaryotes (the origin of mitochondria and plastids) are extremely rare, having occurred only once each in the last four billion years, that is, at the same rate as the origin of life. The origin of eukaryote complexity, which is founded in the eukaryote endomembrane system, occurred at the same rate as the origin of mitochondria (Lane and Martin, 2010).

There are two views concerning the origin of the eukaryotic endomembrane system. In the traditional view, the endomembrane system stems from invaginations of the plasma membrane before the origin of mitochondria. A newer, alternative view has it that the release of outer membrane vesicles from the mitochondrial symbiont to the host precipitated the origin of the endomembrane system from which the nucleus is derived during the cell cycle as well as the origin of bacterial lipids in eukaryotes (discussed in Gould et al., 2016). It is not pure coincidence that the only organelles of eukaryotic cells that we know with certainty to have arisen via endosymbiosis, mitochondria and the plastid family, are bioenergetic organelles. The nucleus, by contrast, is derived from the endoplasmic reticulum (ER), it is not a bioenergetic compartment. The ER is, in turn, derived from vesicles of bacterial-type lipids that stem *inter alia* from the mitochondrion (McBride, 2018). The ER is eukaryote specific because eukaryotes have mitochondria. Some prokaryotes have bacterial endosymbionts, but they do not have

bioenergetic organelles (Lane and Martin, 2010). That is the most sensible reason why prokaryotes remained simple while eukaryotes became complex.

Mereschkowsky did not classify the Saprolegniales as members of the fungal kingdom. Instead, he identified these siphonaceous filaments as plants that have lost their plastids and classified the Oomycota among heterokont algae to which they exhibit the closest affinities. This statement poses the question, however, as to whether diatom plastids can be lost and, more generally, the degree to which symbiosis is a process of evolutionary addition and subtraction. Indeed, the origin of higher taxa via combinatorial processes is nowhere better summarized than the passage from the 1910 paper:

"From this we may set up the following equation:

Diatoms – Plastids = Animals

and from that

Animals + Plastids = Plants."

The second equation is generally correct, the first is problematic as formulated with regard to the diatoms. Mereschkowsky confirmed observations from other scientists that diatoms may lose their photosynthetic pigments as soon as they are cultivated in media containing organic food. But he erroneously concluded that the diatoms lose their plastids altogether. It was only ultrathin sections using diamond knives and transmission electron microscopy that allowed Schnepf (1969) to demonstrate proplastids in permanently apochlorotic diatoms, first in *Nitzschia alba*. Those techniques also uncovered relict plastids among the malaria parasites, which are surrounded by four membranes and harbour a highly reduced plastid genome (McFadden et al., 1996).

For clarity, we have replaced the term Cyanophyceae with cyanobacteria and the term chromatophores with plastids. Our translators' notes and comments are in *[bracketed italics]*, all footnote numbers correspond to the original, as does the use of italics and any emphasis conveyed by increased character spacing. We have indicated the page breaks of the original in brackets, the many footnotes also generate correspondence to the German text. We have corrected minor typographical and bibliographical errors in the footnotes, but have made no corrections to the text. The original is inconsistent with regard to the spelling of mykoid and mykoplasm, sometimes written with c, sometimes with k; the original Greek term μύκης (fungus) is written with κ, which transcribes as k, the convention we have used here. We have rendered species names in lower case throughout, though many are capitalized in the original.

One aspect of Mereschkowsky's lone 1910 figure has gone unnoticed. He depicted the origin of some eukaryotes, namely animals and plants, as involving a physiologically argued serial endosymbiotic mechanism (symbiogenesis), with no gradual intermediates. In the very same figure, however, he depicted the origin of other eukaryotes, the fungi, through a series of stepwise transitions from the first bacteria via haplobacteria (simple forms), trichobacteria (filaments), actinobacteria (endspore forming filaments), and then protomycetes, a hypothetical missing link in a continuous evolutionary grade connecting actinobacteria to the true fungi — a perfectly traditional gradualist transition. That is, not only did he present both sides of a century old debate on symbiogenesis *versus* gradualism for the origin of eukaryotes (Martin, 2017) in the same paper, he summarized both the case for a symbiogenic origin of eukaryotes and the case for a gradualist origin of eukaryotes in the same figure. Ironic would be an understatement.

Today, the host for the first endosymbiosis in eukaryote evolution looks much more like the micrococci in Mereschkowsky's figure than the amoeboid Moneran. According to current data, the host was an archaeon (Imachi et al., 2020), not an amoeboid Moneran. It was a typical archaeon, small and lacking any trace of eukaryotic complexity (Lane, 2020). Though many evolutionary biologists still believe that there was a gradual transition from archaea to Monera like cells of the type Mereschkowsky drew on the left side of his 1910 figure leading to fungi, the data in 2020 has it that the archaeon that is most closely related to the host (Imachi et al., 2020) was a garden variety archaeon, making the prokaryote eukaryote transition steeper than ever before (Gould et al., 2016; Lane, 2020; Speijer, 2020). That brings us to the last words of Mereschkowsky's 1910 paper, which appear in a footnote: "*Either the symbiosis is present, and they are lichens, or the symbiosis is not present, and they are fungi; there are no transitional forms nor can they exist.*" Such is the nature of symbiogenesis.

Declaration of competing interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix

This translation should be cited as: Mereschkowsky C (1910) Theorie der zwei Plasmaarten als Grundlage der Symbiogenesis, einer neuen Lehre von der Entstehung der Organismen. Biol. Centralbl. 30:278–288; 289–303; 321–347; 353–367 (English translation in Kowallik KV, Martin WF. 2020, BioSystems •••, •••–•••). Numbers in brackets, [279] etc., indicate the beginning of the page number in the original.

[Part I, vol. 30, No. 8, April 15, page 278]

The theory of two plasma lineages as the foundation of symbiogenesis, a new principle for the origin of organisms.

by **Prof. Dr. C. Mereschkowsky.**

Preface.

If a problem has not been recognized, it cannot be subjected to investigation. J . R e i n k e .

One of the most interesting and engaging tasks in biological sciences is the question of how organisms originated on Earth.

It is all the more curious that so few have addressed this question. I am not aware of any recent publications on this topic, aside from a few notes and comments that focus on specific minor aspects of the issue.

Previous attempts to solve the problem (D a r w i n , H ä c k e l , N ä g e l i) were necessarily unsuccessful because the observations required to resolve the issue were unavailable at the time. Since then, however, so much new information from cytology, biochemistry, physiology, especially from lower organisms, has accumulated that, supported by new sets of findings, it is worthwhile to renew our efforts to lift the veil masking the secret of the origin of organisms.

Thus I decided to embark upon this endeavour, and the present contribution together with a preceding one[1], as well as a second to come[2] represent a preliminary treatise on a new theory for the origin of organisms, one in which symbiosis plays the major role, for which reason I propose designating this idea as t h e T h e o r y o f S y m b i o g e n e s i s .

The present article is devoted to the fundamental question: how many lineages of plasma *[Plasmaarten]* are there in the organic world? I will try to demonstrate that the entire realm of all living organisms stems from the existence of two so fundamentally different kinds of cytoplasm that the organic world *[organische Natur]* cannot be considered as uniformly homogeneous in its origin and evolution, in contrast to prevailing views.

In principle there are many more than two kinds of cytoplasm. Indeed one could say there are most probably many – one might even say that there are an indefinite number. Each organism that differs from another in some respect also contains a cytoplasm that is specific in some respect. But we have to deal with the question whether these numerous variations [280] reflect modifications of a single or more than one kind of cytoplasm. No one has mentioned this problem to date. Instead, as an implicit agreement everybody accepts the uniformity of the living world. Everybody believes that the foundation of all organisms is only one kind of plasma. In other words: life evolved from the inorganic world by a single root, from which grew a single branched tree of organisms, initially as a common trunk of protists which soon split into two main branches – that of plants and that of animals.

To date everyone is convinced that there is only one tree of life. *[Bis jetzt herrscht die allgemeine Überzeugung, dass der Baum des Lebens ein einziger sei.]* It is the aim of the present paper to demonstrate that there are two trees of life, that the two trees are separate and independent from one another. They probably appeared at different times during the history of Earth, and each grew separately and independently, but to some extent their branches merged and closely intertwined, thereby creating the diversity of the living world.

T h e c o n c e p t o f t h e u n i f o r m i t y o f t h e o r g a n i c r e a l m h a s t o b e a b a n d o n e d i n f a v o r o f t h e i d e a o f i t s d u a l i t y .

January 11, 1909. C.S. v. Mereschkowsky

I. Two lineages of plasma.

To introduce the reader to the sphere of my considerations about the organic world, it seems appropriate to illustrate the material with the help of an image.

[1] M e r e s c h k o w s k y , C., Über Natur und Ursprung der Chromatophoren im Pflanzenreich. Biol. Centralbl. Bd. XXV, 1905, p. 593.

[2] The topic of the paper will be the cell nucleus and in particular the question regarding its nature and origin.

We imagine the following two scenes. First we consider a family gathered for lunch at home. We assume it is summer, outside a sweltering heat of 25–30 °C, the windows are open wide. Food is on the table, milk, meat, eggs, and bread, that the family is eating. The children have finished their meal, are running around the table, the adults are engaged in lively conversation, using their hands to underscore their words. The voices get louder and louder as suddenly a drama unfolds in that a young girl goes to the cabinet, pulls out a bottle of potassium cyanide, drinks it, falls over and expires.

Now imagine a different picture. The same room is covered with a huge bell glass to seal it airtight. All the oxygen is removed from the atmosphere down to the last atom, the air is replaced with hydrogen sulfide, the temperature of the room is increased to over 90 °C. [281] At the table are seated strange creatures, alive but immobile, their food consisting of mineral salts, potassium cyanide, morphine, rubber, chitin, paraffin, antlers…

Given these two images, is it wrong to say that we are dealing with two fundamentally different types of creatures, consisting of substances that are quantitatively and qualitatively different, and that these two substances survive under such different conditions that they can have nothing in common.

Yet these two images that I have just presented are not just imaginary. They exist in reality, in all details, as strange as it may seem. Yet no one has seen these two pictures, or more accurately, everyone has seen them, or has seen them in passing, but no one has noticed them.

Indeed, in nature there exist two plasmas that are so sharply distinct from one another as the family and the strange creatures from the foregoing two images, and each of these plasmas serves as the basis for its group of organisms. *["serves as the basis" reflects the concept of protoplasm as a carrier for the property of life.]* The first plasma gives rise to plants, animals and eventually humans, the second to bacteria, fungi and cyanobacteria.

What then are the differences between the two kinds of cytoplasm? The table below, which summarizes the more prominent differences only, demonstrates how numerous the differences are and how fundamental they are.

Mykoid plasma. (Mykoplasma)	Amoeboid plasma. (Amoeboplasma)
1. Can live without oxygen (bacteria).	1. Cannot live without oxygen.
2. Withstands temperatures beyond 90 °C and higher (bacteria, cyanobacteria).	2. Does not tolerate temperatures higher than 45 to 50 °C.
3. Able to synthesize proteins from inorganic substances (bacteria, fungi, cyanobacteria, plastids).	3. Not able to synthesize proteins from inorganic substances, requires organic food.
4. Incapable of amoeboid movement, unable to form pulsating vacuoles (bacteria, fungi, cyanobacteria, plastids, nuclei).	4. Capable of amoeboid movement, creates pulsating vacuoles.
5. Rich in phosphorus and nuclein (bacteria, fungi, nuclei).	5. Lacks large amounts of phosphorus, does not contain nuclein.
6. Hydrogen cyanide, strychnine, morphine are metabolized. Very robust.	6. Hydrogen cyanide, strychnine and morphine are toxic substances Less robust.

I propose to designate the second kind of cytoplasm, which is fundamental to plants and animals, as A m o e b o p l a s m a , [282] because its typical characters emerge most prominently in the amoeba, where it is in strong movement, sensitive to even the slightest lack of oxygen as well as to minimal concentrations of toxic substances and which is only able to live on preformed food like proteins and carbohydrates.

Fundamentally different from this is the plasma that serves as the basis of the m y k o i d s — the term I use to collectively designate the bacteria, fungi, cyanobacteria, including the plastids living symbiotically within the amoeboplasma, as well as of certain components of the cell nucleus. This immotile form of cytoplasm is rough, crude, robust and independent, with strong and rigid character. It can withstand the most hostile environmental conditions imaginable (see Chapter VII), it is not picky about food sources, it synthesizes its own proteins and can live from toxic substances that are lethal to the amoeboplasma even in the smallest amounts. It carries the imprint of the harsh conditions that no doubt existed on the young Earth at the time that this plasma emerged. Therefore I propose to designate this kind of plasma as M y k o p l a s m a .

It is possible that I will be confronted with the criticism that I use a term previously introduced by E r i k s s o n to describe a specific hypothetical cytoplasm by which certain fungi (*Uredineae*) hibernate inside the seeds of higher plants, thereby again starting their life cycle during the spring to come. But the existence of such a cytoplasm has never been proven to date, and no one believes in it; maybe it does not exist at all[3]. As a

[3] W a r d demonstrated that the bodies that Eriksson considered to be the initial secretion of fungal cytoplasm appearing to merge with the plant cytoplasm reflect nothing other than haustoria of the fungal hyphae extending into the host's cytoplasm, which nourishes the fungus. W a r d , Marshal, On the histology of *Uredo dispersa* (Eriksson) and the Mykoplasma hypothesis. Proc. Roy. Soc. Vol. 71, 1903, p. 355 and Philosophical Transactions Roy. Soc. London. Ser. B, Vol. 196, 1903, p. 29–46.

consequence one may consider this term as free for use. Therefore and according to the traditional judicial idiom "res nullius cedit primo occupanti" *[An unowned thing belongs to its first possessor.]* I have decided to use the term for this new concept, all the more in that a more appropriate term is hardly imaginable. *[Eriksson responded kindly in September, Biol. Centralbl. 30:618–623 (1910).]*

Let us now consider, in more depth, all six main differences between mykoplasma and amoeboplasma, which have been summarized in Table 1.

II. Respiration.

Oxygen respiration, as it is widely known, is one of the most important conditions for animal and plant life. [283] According to Pfeffer[4], "respiration never comes to its end as far as the general conditions for life are fulfilled; it even continues independently in dormant organs like onions, bulbs etc."

The protoplasm of animal and plant cells (amoeboplasma) is not able to survive without access to oxygen. No plant or animal can survive without oxygen[5] such that "the interruption of respiration can be considered the unerring proof of death"[6]. Typically after 5–10 minutes, almost always after few hours — in rare cases longer, all organisms will die in an oxygen-free environment. Although in certain cases the typical respiration may be replaced by the removal of oxygen from other substances (Spaltungsatmung) *[Spaltungsatmung is an archaic term for fermentations]*, that is by so-called intramolecular respiration like in fruits and other plant organs[7], or in some parasitic worms[8], one cannot consider this kind of respiration to be anything other than pathological[9], because in the end any organism will inevitably die following absence of oxygen.

[284] "Without free oxygen no life can permanently exist", says Verworn[10].

A miraculous exception to this rule are the bacteria — one of the members of the mykoids. Some bacteria are able to live indefinitely without oxygen as first shown by Pasteur. And this capability is not only possessed by a few bacteria, but by many of them. Bacteria that not only live without oxygen, but do not even tolerate it, i.e. the so called obligate anaerobes, are widespread, and even more numerous are the so called facultative anaerobes. What do Schmidt and Weis[11] say about bacteria that live without oxygen: "We currently know a large number of bacteria that live in the same manner (that is, without oxygen) as well as an even larger number that grow under suitable culture conditions with or without oxygen. By comparison there are relatively few that specifically require free oxygen for growth. Because the bacteria are the only group of organisms known so far that can live continuously without oxygen, they assume a special place among life forms."

The fact that there are organisms able to live without free oxygen seems so unusual to some experts that they doubt whether such organisms truly exist that can survive without obtaining energy from oxidative processes. — Some tried to explain this phenomenon in different ways in order not to infringe upon with the rules that generally apply for all living organisms. Especially Beijerinck[12] suggested that during anaerobic growth such bacteria might live from oxygen that they had accumulated and sequestered within their cells during aerobic growth phases. Other attempts to explain this phenomenon were put forth. According to Schmidt and Weis[13] "None of those explanations appear to be correct; in contrast, there can be no doubt that there are indeed bacteria that can live and propagate for an unlimited number of generations in media where oxygen cannot be demonstrated even by most sensitive experimental assays."

Therefore the bacterial plasma is able to live without oxygen, the amoeboplasma is not. This difference is extremely important and has fundamental significance. [285] Most probably, both plasma lineages are fundamentally different in their chemical composition and behaviour. — Schmidt and Weis (loc. cit.) consequently come to the fully justified conclusion that "anaerobic bacteria must live in a manner that is totally different from that in aerobic bacteria"; we do not have to contrast anaerobic bacteria just with aerobic bacteria, but also with all animals and plants collectively. The plasma of anaerobic bacteria must live in a manner that is totally different from that of animals and plants.

If one takes into account that the majority of bacteria belongs to the anaerobionts and that most bacteria are able to live without oxygen and can gradually adjust to anaerobic

[4] Pfeffer, W., Pflanzenphysiologie Vol. I, *[sic:* Leipzig, 1897*]* p. 523.

[5] Dude, M., Über den Einfluss des Sauerstoffentzuges auf pflanzliche Organismen. – Flora, Vol. XCII, 1903, p. 205. According to Kühne, among the plants, only *Nitella* can survive for about a month without oxygen (Kühne, W., Über die Bedeutung des Sauerstoffes für die vitale Bewegung. Zeitschr. f. Biologie, Vol. 36, 1898, N.F., Vol. 18, p. 1), although Ritter could not confirm this observation. He found that this plant could only survive a few days without oxygen (Ritter, Abhängigkeit der Plasmaströmung und Geißelbewegung vom freien Sauerstoff. Flora, Vol. 86, 1899, p. 329). With regarding to animals see Pütter, A., Die Atmung der Protozoen. Zeitschr. f. allgem. Physiologie, Vol. V, 1905, p. 566. – The parasitic ciliates *Opalina* and *Balantidium* can survive 9–13 days in protein without oxygen, *Nyctotherus* even 50 days, *Spirostomum* only 32–48 hours and *Paramaecium* 4–240 hours depending on the circumstances.

[6] Pfeffer, W., Pflanzenphysiologie Vol. I, *[sic:* Leipzig*]* 1897, p. 523.

[7] According to Palladin, the carbonic acid produced during intermolecular respiration is mainly nuclear carbonic acid, which is one caused by enzymes which are probably products of the cell nucleus (Palladin, Über den verschiedenen Ursprung der während der Atmung der Pflanzen ausgeschiedenen Kohlensäure. Ber. der deutsch. botan. Gesellsch., Vol. XXIII, p. 240). If this is the case, the difference between mykoplasma and amoeboplasma in terms of the need to breathe becomes even more evident.

[8] The latter can live in a completely oxygen-free medium for 4–5 days without stopping their vigorous movements. Bunge, Über das Sauerstoffbedürfnis der Darmparasiten. Zeitschr. f. physiol. Chemie, Vol. 8, 1883.

[9] This is already evident, as Godlewsky (Botan. Centralblatt, 1906, p. 539) has shown, from the fact that in intracellular respiration, only processes of dissimilation of proteins which produce asparagine are possible when the protein is destroyed. A synthetic formation of asparagine as the beginning of the regeneration of proteins does not take place in higher plants.

[10] Verworn, M., allgem. Physiologie. Jena 1901.

[11] Schmidt, Johs und Weis, Fr., Die Bakterien. Jena 1902, p. 133 and 134.

[12] Beijerinck, M., citied in Schmidt and Weis 1. c.

[13] Schmidt and Weis, 1. c., p. 136.

conditions[14], one then has good reason to assume that anaerobiosis was the first and most ancient *[erstälteste, primordial]* bacterial condition. Hence it follows that the urbacteria or protobacteria already existed at a time during the history of Earth when our planet was covered with boiling water, which as a consequence *[of boiling]* could not contain dissolved oxygen. *[He had anaerobic origins right but for the wrong reason.]* — The initial plasma that appeared on Earth was of such nature that its growth did not require free oxygen, which could not have been present in ancient hot water. From their primordial origin, bacteria were therefore anaerobes. Only later, when the Earth's temperature has fallen so that water could contain oxygen in dissolved form, some bacteria began to adapt to the new conditions, thereby becoming facultative anaerobes eventually followed by a few obligate aerobes. The fact that the latter can be transformed into anaerobic bacteria again appears to offer important confirmation for the correctness of the aforementioned view. When the fungi evolved from the bacteria[15] *[a recurrent error]* the plasma of these organisms, which primarily living in contact with air [286], adapted fully to life in oxygen rich environments. However, from the circumstance that fungal plasma's requirement for oxygen is an adaptation, one may not conclude that the mykoplasma changed its structure in such a way as to become identical with that of the amoeboplasma. Properly speaking, the oxygen respiration of certain bacteria and of fungi cannot be taken as proper proof that it is identical with that of the amoeboplasma, irrespective of its initial and final steps (uptake of oxygen and exhalation). All steps in between may be identical but can also be totally different. *[Convergence of respiration, see footnotes 15 and 16.]* As we may see in the following that there are additional differences between the amoeboplasma and the mykoplasma, which are conserved in the kingdom of fungi, such that we may eventually find the individual steps of respiration to reveal specific characters in fungi which discriminate them from those of the amoeboplasma[16]. [287]

III. Relationship to temperature.

Kühne[17] was the first to start with extensive investigations regarding the behaviour of amoebae, infusoria, and diverse tissues against extreme temperatures. From these experiments with maximum temperatures that are tolerated by various lower animals as well as plants, one could learn that even at 35 °C amoebae, which below that temperature exhibit vigorous movement, lost this ability in that they contracted their bodies but remained alive. Raising the temperature up to 40 and 45 °C and subsequent cooling down did not bring them back to life. Kühne was able to demonstrate differences between individual plasmas in relation to temperature. The contractile plasma coagulated already at 40 °C and disintegrated, the remaining part of the plasma at 45 °C. Max Schultze[18] found that plant cells withstand 47 °C, beyond that they died[19]. Since then many observations were made with respect to the resistance of various organisms to high temperatures which are summarized in tables of Fürth[20] and Davenport and Castle[21] which served as material for the following brief compilation.

[14] Willimsky, W., Arch. f. Hygiene. Vol. LIV, Issue 4, 1905.

[15] The fungi, as we will see in the further course of our treatise of the theory of symbiogenesis, are much more closely related to the bacteria, with which they share much in common, than they are to any other organisms. For this reason they can be considered as having evolved from bacteria. — Among the latter we can already observe the primordial beginnings of the characters, which in their further development lead to the formation of the two groups of fungi, Ascomycetes and Basidimoycetes. In fact, if we look carefully at the way spores are formed in bacteria in general and in *Bacillus bütschlii* in particular (Schaudinn, Fr., Beiträge zur Kenntnis der Bakterien und verwandter Organismen. I *Bacillus bütschlii* n. sp., Arch. f. Protistenkunde. Vol I, 1902, p. 306), we must conclude that there is a fundamental correspondence in the way spores are formed in both groups. With *Bacillus bütschlii*, we can say that this process is identical to the process of spore formation in Ascomycetes and the bacteria themselves are not analogous with the ascus of the fungi, but homologous. *[This is incorrect, they are not homologous.]* It is an ascus that forms two spores. On the other hand some observations from the life of the bacteria are reminiscent of the formation of conidia. In the Actinomycetes, which some group among the bacteria while others group them among the fungi, there are already visible and fully developed conidia. In this manner, two of the most characteristic features of the fungal organization, the ascus and the conidia, are already present in their ancestral manifestation in the bacteria. It must be added that in both bacteria and fungi the cell wall consists of nitrogenous substances and that neither one nor the other shows any trace of amoeboid movement of the protoplasm, that zoospores and contractile vacuoles are completely absent in both, etc. — A. Meyer has recently taken a similar position (Meyer, A., Studien über Morph. u. Entwickelungsgesch. der Bakterien. Flora, Vol. 84, 1907, p. 240).

[16] The respiration process of fungi is still so little studied that it cannot even be claimed that all fungi need oxygen to breathe. If one keeps in mind that there are fungi that are able to fix free nitrogen from the air (see below), much like some bacteria do — something no amoeboplasma can do — and that furthermore fungi have the ability to synthesize protein molecules, it seems clear that fungi differ so much in physiological aspects from amoeboid organisms that it would not be surprising if the middle phase of fungal respiration turns out to be different from that of amoeboplasm. It is very probable that some fungi, especially those capable of fixing free nitrogen, would prove capable of living completely without oxygen, that is they would become as anaerobic as the majority of bacteria. This is all the more probable because, in Beijerinck's opinion (Beijerinck, M., Botan. Centralbl. 1905, p. 298), the ability of bacteria capable of fixing free nitrogen is always related to the ability to live without oxygen. If this is the characteristic of the bacteria, it will probably also be applicable to the fungi. Experiments in this direction would therefore be extremely desirable.

[17] Kühne, Untersuchungen über das Protoplasma und die Kontraktilität. Leipzig 1864.

[18] Schulte, Max, Das Protoplasma der Rhizopoden und der Pflanzenzellen. Leipzig 1863.

[19] According to Miehe "for all higher plants and for most lower ones, one can assume that their ability to survive is restricted to the range 0–10 °C and 35–45 °C … For metazoans the maximum temperature usually resides beween 30 and 40 °C, it rarely reaches 45 °C, so that the survival for the majority of life forms is excluded above 45 °C." Miehe, H., Die Selbsterhitzung des Heues. Jena 1907, p. 89.

[20] Führt, Otto, Vergleichende chemische Physiologie der niederen Tiere. Jena 1903, p. 424

[21] Davenport und Castle, Studies in Morphogenesis. III. On the acclimatisation of organisms to high temperatures. Arch. f. Entwickelungsmechanik, Vol. II, 1896, p. 227.

[287-288]

Table of maximum temperatures in amoeboid organisms.

Animals.	Maximum °C	Plants.	Optimum °C	Maximum °C
Aethalium septicum	40	Mucor racemosus	20—25	33
Amoeba	40—45	Mucor pusillus	—	50
Actinophrys	42	Spirogyra	—	44
Several flagellates	60[22]	Cladophora	—	45—60
Carchesium	47	Oedogonium	—	44
Stentor	44—50	Ulothrix zonata	15	24
Actinia	38	Vaucheria repens	—	30
Beroë ovatus	40	Hydrurus foetidus	10	16
Cestus veneris	34	Plant cells according to		
Medusa	36—39	Schultze	—	47—48
Various molluscs	30—40	Triticum vulgare	29	42
Aplysia	33	Sinapis alba	27	37
Antedon	30	Acer platanoides	24	26
Urchins	39—41	Pinus silvestris	27	34
Vinegar nematodes	30	Phaseolus multiflorus	34	46
Turbellaria	45	Zea mais	34	46
Anguillulidae	45	Cucurbita pepo	34	46
Terebella	27—30	Elodea canadensis[23]	—	42
Daphnia sina	34			
Cyclops quadricornis	36		(To be continued.)	
Gammarus	36			
Palaemon	26			
Culex pipiens (larva)	40			
Hippocampus	30			
Frog	44			
Salamander	44			
Dog	44—45			

[end Part I, vol. 30, No. 8, April 15, page 288; begin Part II, vol. 30, No. 9, May 1, page 289]

(Continuation).

The table on p. 288 demonstrates that in the vast majority of cases the amoeboplasma cannot survive at 45 °C, at maximum 50 °C. Only in few cases some flagellates survive up to 60 °C[24].

[290] Totally different is the behaviour of the mykoplasma at higher temperatures.

Oscillatoria species (belonging to the cyanobacteria), for example, were able to tolerate 64.7 °C, and according to careful observations made in America at the hot springs of Yellowstone National Park by Hoppe-Seyler[25], these algae[26] were found alive at even higher temperatures. *Hapalosiphon laminosus*, another cyanobacterium, lived in water above

[22] At this temperature death did not yet occur; the movement continued even at 52° C. (Hauptfleisch, Jahrb. f. wiss. Botan., Vol. XXIV, 1892, p. 209), but how long the plant remained under the influence of this temperature is not listed.

Completely dry seeds of *Pisum sativum* can tolerate a temperature of 70° C. without losing their ability to germinate, but not for more than one hour (Sachs, J., Lehrbuch der Botanik, 3. Edition, Leipzig 1873, p. 639). — Here, however, we are dealing with a body of immense size in relation to the bacteria or fungal spores. If the spores of the bacteria were the size of a pea, it is very likely that they would withstand a much higher temperature. The temperature limits of the seeds are therefore not comparable with those of bacteria. See also Schubert, Flora, 1909, p. 68.

[23] Dallinger, W. H., On a series of experiments made to determine the thermal deathpoint of known Monad germs, when the heat is endured in a fluid. Journal Royal Microsc. Soc. 3, 1880, p. 1–16. – Unfortunately, I did not have the opportunity to acquaint myself with the original work, and so I cannot judge the credibility of the facts cited by Dallinger. Doubts are mainly raised by the question what this author understands under the terms Monad Germs, that is, whether he really worked with amoeboplasma all the time or whether he actually had mykoids among those monads. In the latter case, the high temperatures that his "spores" in particular are capable of withstanding would be understandable.

[24] A few highly questionable cases of the survival of animals in hot springs with even higher temperatures have not been included among the present observations due to the low level of detail provided in nature of such reports. In fact, it remains unclear whether the temperature in the whole spring remains at the same high temperature or whether there are more cooler places in which these animals could usually live, only passing over into the hot places for a short time. Furthermore, we have the very carefully executed experiments by Dallinger (Dallinger, The President's Address. Journal Royal Microsc. Soc. 1887, p. 185–199) on protozoa, the maximum temperature of which was 60°; by acclimating them to higher and higher temperatures over the course of a few years, he succeeded in raising a breed capable of withstanding temperatures up to 70°. – But this fact must also be ignored here, as this artificial resistance cannot be compared to the organism's normal relationship with high temperatures. In fact, if Dallinger had succeeded in raising the maximum temperature by 10°, then perhaps if he had applied the method of gradual habituation to bacteria or cyanobacteria, he would have succeeded in raising these organisms to a higher maximum temperature as well, and only then would it have been possible to compare the two numbers. – Now the height of the exceptionally high maximum found by Dallinger and the value of the normal temperature for other organisms not yet accustomed to the temperature is not comparable. From the above it is clear how important it is to perform experiments similar to those Dallinger made with protozoa, but using bacteria and cyanobacteria as a reference system.

[25] Hoppe-Seyler, Physiol. Chemie. Part I, Berlin 1877.

[26] The cyanobycteria, although belonging to the mykoid family, are called algae because of their green colour, which are still today grouped among them by some.

90 °C[27]. Went also states that "the highest temperature at which filamentous Myxophyceae *[archaic]* (that is, cyanobacteria) are known to exist is 86 °C" [28] and adds "but unicellular algae have been observed by Brewer in California at 94.4 °C"[29].

These observations were frequently disputed[30], but for no good reason; at least some of these reports can be taken as absolutely valid when one reads the reports of similar observations made by de Vries[31]: "The water reaches almost boiling temperature near the edge. In some springs I measured temperatures of 86–90 °C while the bulb of the thermometer was pressed against the algae". [291]

There is no doubt at all that cyanobacteria are able to live and propagate at temperatures of 86–90 °C or even 94.4 °C, that is, in almost boiling water.

Observations of this kind and of no less astounding nature come from the bacteria, another group of the mykoid kingdom.

While most of animals and plants will die at temperatures above 40–45 °C, there are bacteria that stop living at temperatures lower than that, they prefer temperatures of 60–70 °C — their temperature optimum — where they divide most rapidly[32].

And thus we see that water at 70 °C, in which every crustacean, every fish, every vegetable will die, water that would scald every hand dipped into it, is at the optimum for mykoid plasmas. — Occasionally bacteria may multiply even at 75 °C at which temperature any protein known to us will coagulate. Yet Miehe[33] and Karlinski[34] observed bacteria (*Bacterium ludwigii, Bacillus calfactor, Bacillus ilidsensis capsulatus*) living at 80 °C!

Among those *Bacterium ludwigii* is completely unable to survive below 50 °C, a temperature at which all animals and plants would have long since expired!

These to the highest degree remarkable bacteria, with which we are dealing here, are designated as thermophilic bacteria *[thermophile Bakterien]* or simply thermobacteria. — One encounters them frequently in the uppermost soil layer that is exposed to the heat of the sun, in warm springs, in excrements and decomposing organic matter where due to fermentations the temperature may increase dramatically, sometimes inside the intestine of humans and animals where, according to Rabinowitsch[35], they may live at temperatures lower than usual, due to the absence of oxygen. [292]

But even these observations do not set the extreme limits of thermotolerance that characterize bacteria, as may be deduced from Eisenberg's[36] most recent observations on *Bacillus anthracis*. When these bacteria are subject to 70 °C for 15 minutes numerous individuals in their vegetative state tolerated this high temperature and were able to propagate. Following 80 °C for 15 minutes the number of living bacteria decreased, but even after treatment at 90 °C for 5 minutes a few bacteria remained alive and were able to multiply. Eisenstein was convinced that it was not the spores of that bacterium which survived this high temperature. He obtained similar results for the oidia *[archaic for conidia or arthrospores]* from a few cultures that resisted 98 °C for 15 minutes. Similar results he obtained from the soil bacterium *Bacillus tumescens* and two other bacteria: *Bacillus megatherium* and *B. ramosus liquefaciens*.

Even more astounding is the behaviour at high temperatures of bacteria in their resting state as spores. Koch, Brefeld and others showed that the spores of *Bacillus anthracis* as well as the spores of the hay bacterium *Bacillus subtilis* are able to withstand 100 °C and more without losing their ability to live. Especially resistant in this respect are some soil bacteria which occasionally contaminate the milk of dairy cows. Christen[37], for instance, found forms of which that can be destroyed under hot vapor and high pressure when autoclaved at the following temperatures dependent upon the duration of the steam treatment.

Even more resistant proved to be spores reported by R. Koch and Wolffhügel that survived in air stream heated up to 140 °C for 3 hours and were able to withstand 150 °C for about one hour with no damage[38].

[293] That is, there are bacterial spores that can survive almost an hour at 150 °C and remain viable! Verworn[39] says "To date we have no plausible explanation for this mysterious phenomenon. We can only suggest that inside these organisms reside proteinaceous compounds that cannot be brought to coagulation by high temperatures".

Attempts were made to explain this remarkable resistance against high temperatures not with specific characters of the protoplasma, but with the protecting properties of the unusual

[27] Schmidt, Johs und Weis, Fr., Die Bakterien. Jena 1902, p. 144.

[28] West, G. S., Some Algae from Het *[sic, Hot]* Springs. Journal of Botany. July 1902, p. 241.

[29] West, G. S., A Treatese *[sic]* on the Freshwater Algae. 1904, p. 307, in which Brewer's paper is quoted. Brewer, W. H., American Journal of Science. Ser. 2, Vol. XLI.

[30] The doubts were based on the possibility of large temperature differences between two points close to each other in the hot springs; consequently, if the thermometer was not carefully set at the same place where the algae grew, a mistake is entirely possible. De Vries's observations, as we will see shortly, remove all doubts.

[31] De Vries, H., Arch. Néerland. V, 1870, p. 385. The quotations are taken from Lotsy (Vortrag über botan. Stammesgeschichte I, p. 374).

[32] It would be extremely interesting to conduct experiments on the robustness of plastids (especially in lower plants) to high temperatures. If the plastids are observed outside and inside the cells, it might be possible to show that the plastids are able to withstand higher temperatures than the amoeboplasma using Engelmann's bacterial method to reveal the assimilation activity of plastids.

[33] Miehe, H., Die Selbsterhitzung des Heues. Jena 1907.

[34] Karlinski, Zur Kenntnis der Bakterien der Thermalquellen. Hygien. Rundschau Vol. 5, 1895, p. 685.

[35] Recently it has been shown that Rabinovich's opinion is unfounded, that is, that the presence of oxygen does not have the effect that Rabinovich ascribes to this factor.

[36] Eisenberg, P., Über die Thermoresistenz der vegetativen Formen der aeroben Sporenbildner. Centralbl. f. Bakterien (Abt. I), Vol. XCVII, 1908, p. 187.

[37] Schmidt, Johs u. Weis, Fr., Die Bakterien. Jena 1902, p. 155.

[38] Lafar, Fr., Handbuch der technischen Morphologie. Vol. I, Jena 1905, p. 447.

[39] Verworn, Max, Allgemeine Physiologie. Jena 1901, p. 305. Such observations seemed so unlikely that people refused to believe them for quite some time. Sachs states: "Diverse new reports about the high temperatures that fungal spores can withstand without losing their viability are hardly to be believed, and require critical reevaluation to such a degree that I will simply disregard them here". Sachs, J., Lehrbuch der Botanik. 3. Edition, 1873, Leipzig, p. 639.

strong and impermeable envelope which surrounds the spores. But there is no envelope that is able to protect the interior of a tiny spore against such high temperatures applied for an hour[40]. That would contradict all laws of physics. And finally one has to take into account that not only spores, the resting stages of living organisms, may tolerate unusually high temperatures like 100 °C, but also living organisms in their vegetative state, being able to grow and multiply both among bacteria and cyanobacteria.

Obviously, we are faced with a kind of protoplasma that is of a different nature from that of the amoeboplasma. Therefore, Pfeffer[41] is correct when he states: "it is striking that thermophilic bacteria which grow well at 74 °C (we have seen that they even live at 94 °C) or spores which in a hydrated condition withstand boiling temperature for 30 minutes[42], do not contain such proteinaceous compounds coagulating already at lower temperatures."

At	100°	only	after	16	hours
"	105—110°	"	"	2—4	"
"	115°	"	"	30—60	minutes
"	120°	"	"	5—15	"
"	125—130°	"	"	about 5	"
"	140°	"	"	about 1	"

Attempts have also been made to explain the resistance of the spores of certain organisms against high temperatures with the consistency of their cytoplasm which appears more dense, that is, it contains less water and is therefore, so to speak, more dry. [294] — And indeed, as Lewith[43] has shown, the coagulation temperature rises considerably with decreasing water content of the protein.

Pfeffer[44], in contrast, does not agree with such explanations. He states: "Since this kind of resistance is also due to recently formed spores, and not just those taken from culture medium, which are undoubtedly water saturated, the resistance cannot result from dehydration as Cohn[45] and some other researchers (Cramer, Davenport) suggest." According to Pfeffer, death in this case is not caused by the coagulation of proteins, all the more because not all proteins are subject to coagulation.

However, even if one holds that the aforementioned explanations for the tolerance to high temperatures are correct, it does not diminish the magnitude of differences between amoeboplasma and mykoplasma regarding their behaviour to temperature: whereas the mykoplasma is able to increase its density in a way that the amoeboplasma cannot, this character reflects important differences distinguishing the two plasma lineages. These capabilities allow the one plasma to markedly increase its density, making it extremely resistant against high temperatures, whereas the other plasma, which is unable to compress itself, remains sensitive and delicate in this respect[46].

So far, we have considered the relationships of two groups of mykoids, the cyanobacteria and bacteria, to temperature. With respect to fungi, the manifestation of convergence *[Konvergenz]* gradually led the mykoplasma, of which also the fungi are composed, to a more or less close similarity to the life properties of the amoeboplasma. Under the influence of their parasitic or saprophytic lifestyle, the plasma of fungi relinquished some of its robustness *[hat sich verzärtelt]* [295] although the unmistakable imprint of the original rough, crude mykoplasma, the bacteria, from which the fungal plasma descended, can still be discerned.

Tsiklinsky[47], for instance, detected a filamentous fungus living in both the soil as well as forming a cotton-like felt on bread, which can grow up to 60 °C. A similar mould was observed by Behrens[48] upon damp millet seeds. Tsiklinsky also found two species of *Actinomyces* of which one grows very frequently in soil, dung, hay, straw, potatoes etc., which was named by her as *Thermoactinomyces vulgaris*; it grows best (optimum) at 57 °C and reaches its maximum only at 70 °C. The spores of this fungus survive in humid heat at 100 °C for 20 minutes.

In the table below, which does not claim to be complete, I have compiled some observations that appeared to me as being of specific interest regarding the robustness of the mykoplasma towards high temperatures when compared with a similar compilation shown in the table for amoeboplasma (see page 287 f.).

[40] Migula (see Lafar, Techn. Mykologie, Vol. I, p. 116) states that this "view is certainly incorrect" and attributes the resistance of bacteria to such high temperatures to the properties of their protoplasm.

[41] Pfeffer, W., Pflanzenphysiologie, Vol. I, Leipzig 1897, p. 54.

[42] In reality, as we have seen, there are spores that, when wet, can withstand a temperature of 150 °C for one hour!

[43] Lewith, S., Über die Ursache der Widerstandsfähigkeit der Sporen gegen hohe Temperaturen. Arch. f. experim. Pathol. u. Pharmakol. Vol. 26, 1890, p. 351.

[44] Pfeffer, W., Pflanzenphysiologie, Part II, Leipzig 1904, p. 294.

[45] Cohn, F., Beiträge zur Biologie der Pflanzen, Vol. 2, 1887, p. 266.

[46] It is possible that both properties of mykoplasma – the ability to resist high temperatures and the inability to perform amoeboid movements – appear as a result of some general characteristic of the physical structure of this plasma. Possibly the general cause of these two phenomena lies in the high density of the mykoplasma. — I urge those who like to get to the essence of things à la Nägeli to think up two micellar theories, one for amoeboplasma and the other for mykoplasma, which should be constructed in such a way that the latter would simultaneously explain the ability of mykoplasma to tolerate high temperatures and its inability to move.

[47] Tsiklinsky, P., Sur les Mucédinées thermophiles. Annales de l'Instit. Pasteur. Vol. XIII, 1899, p. 500 and the same : Sur les microbes thermophiles des sources thermales, l. c., p. 788.

[48] Behrens, J., in Lafar, Handbuch der technischen Mykologie, Vol.I, Jena 1905, p. 449.

[295–296]

Table of maximum temperatures in mykoid organisms[49].

	Optimum °C	Maximum °C	Observer
Bacillus ilidsensis capsulatus	—	80	Karlinski
Cladothrix	55	65	Kedzior
Bacille No. 2	58—60	70	Tsiklinsky
Bacille No. 3 et 4	68—71	73(?)	Tsiklinsky
Mould on bread	56	?	Behrens
Thermomyces lanuginosus	54—55	63	Tsiklinksy
Thermoactinomyces vulgaris	57	70	Tsiklinsky
Whose spores tolerate 20 min in steam	—	100	Tsiklinsky
Streptothrix	55	62	Sames
Bacillus ramosus	25—28	38	W. Ward
Aspergillus fumigatus	38—40	60	Rénon, Cohn
Aspergillus lignieresi	—	53	Constantin et Lucet
Aspergillus micro-virido-citrinus	—	45	Constantin et Lucet
Thermophilic bacteria	60—70	75	Globig, van Tieghem, Same
Thermoidium sulphureum	35—45[50]	53	Miehe (Ber. d. dt. bot. Ges. 1908)
Bacillus subtilis	—	50	Brefeld, Schreiber
Bacillus, gradually accustomed to	—	58	Tsiklinsky
Bacillus subtilis, spores 25—30 min	—	140	A. Meyer
Ustilago carbo, spores, dry	—	104—120	Hoffmann
Ustilago destruens, alike	—	104—120	Hoffmann
Oidium aurantiacum, alike	—	140	Payen
Penicillium glaucum, alike	—	127—132	Pasteur
Peziza repanda	—	138	Schnitz
Cyanobacteria	—	86—90	De Vries
Cyanobacteria	—	85	Went
Cyanobacteria	—	94.5	Brewer
Hapalosiphon laminosus (Cyan.)	—	90	Schmidt & Weis, Bakt. 144
Oscillaria	—	64.7	Hoppe Seyler
Saccharomyces	28—34	34—40	Pedersen, Hansen
Saccharomyces	—	60—65	Kayser
Saccharomyces, dry	—	115—120	Manassein
Saccharomyces, dry	—	75—80	Zopf
Thermoascus aurantiacus	—	55	Miehe
Spores of bacteria, moist, 1 min	—	140	Christen
Spores of bacteria, for 1 hour	—	150	Koch, Wolffhügel
Bacterium ludwigii	55—57	80	Karlinsky
Bacillus calfactor	—	65—70—80	Miehe
Bacillus anthracis, vegetative. State of sporulation, 15 min	—	70	Eisenberg
Bacillus anthracis, alike, 5 min	—	90	Eisenberg
Bacillus anthracis, alike, Oidien, 15 min	—	98	Eisenberg
Bacillus tumescens Zopf, vegetative	—	70—98	Eisenberg
Bacillus megatherium, vegetative	—	70—98	Eisenberg
Bacillus ramosus liquefaciens, vegetative	—	70—98	Eisenberg

[296] The presence of organisms that are able to live and propagate near boiling temperature is a most important character from the theoretical point of view. We have already seen in our considerations about respiration that the first organisms on Earth, bacteria, appeared during a period when the water was still boiling and consequently could contain no oxygen. In order to make the origin of life during that period possible, one has to accept that not only the absence of oxygen in the water, but also the very high temperature did not impair the emergence of life. And now we can be confident that even temperatures at the boiling point, or close to it, do not present an obstacle. [297]

IV. The Synthesis of Proteins.

The mykoplasma synthesizes proteins from simple inorganic substances —, an ability that the amoeboplasma lacks

[49] It should be noted that some maximum values here refer to growth, but since organisms can tolerate temperature increases after growth has stopped, while remaining alive, some of the values given in this table must in fact be even higher.

[50] Still grows well at 50 °C.

[51] Centralbl. für Bakt. (Abt. I), Vol. XCVII, 1908, p. 187. *[Footnote 51 is missing in the text, likely belongs in the table. 1908 is vol. XLVII, page 187 occurs in a paper by Hottinger about Bacillus suipestifer, but not on max. temperature.]*

altogether. Therefore the latter can only exist and propagate if it is provided with proteins from outside[52].

That this sentence is true as it applies to the animal kingdom is self-evident. But it is also fully applicable to plants since the cytoplasm of plant cells is unable to synthesize organic substances from inorganic ones, even simple carbohydrates. Like the animal cytoplasm, it is dependent upon organic food sources.

This sounds somewhat paradox as plants were usually considered as typical representatives for autotrophic life. Nevertheless, the sentence is absolutely correct because plants, if they synthesize organic substances from inorganic ones, can do this only by virtue of the plastids that they harbour; these supply the plant cell with organic food, as plant cells are incapable of synthesizing complex organic substances on their own[53]. The plastids, as already set forth elsewhere, are not germane to the plant cell itself[54]. [298] — Indeed, there exist strong arguments not to interpret plastids as organs or organoids of the cell that plant cells generate endogenously, but as specific organisms of the mykoid kingdom that intruded, from outside, the cytoplasm of an animal cell, with which they became arranged into the intimate symbiosis that we now designate as "plants". And precisely these organisms (cyanobacteria) that entered from outside the cell, are manifest as the internal supplier of the organic substances to the plant cell. They, and not the plant cell itself, are observable in this way as autotrophic organisms. The plant cell breathes and obtains nourishment like any animal cell, albeit with the difference that animals obtain their food from outside whereas the plant receives food from the inside, by virtue of its possession of internal producers of organic substance.

In this way, neither the amoeboplasma of animals nor that of plants is able to produce complex organic compounds like carbohydrates, amino acids or proteins[55].

Totally different is the situation for the mykoplasma. With the greatest of ease it synthesizes the most highly complex organic molecules from the simplest inorganic precursors. We can demonstrate this with few examples.

The Bacteria.

Bacteria are capable to assimilate nitrogen from air with the aid of mineral salts and water to produce proteins for their own protoplasma. Such capabilities are known for *Bacterium*, or as it is more commonly called, *Clostridium pasteurianum*. According to the experiments of Winogradsky[56], this soil bacterium can grow and multiply in liquid media totally lacking any nitrogen containing substances, whether they are of organic or inorganic nature, but merely on the basis of sugar. [299] Hence it follows that such bacteria receive the nitrogen, from which they build their proteins, from the nitrogen of the atmosphere. In addition to the findings of Winogradsky other soil bacteria have been found that are able to bind atmospheric nitrogen. As an example, Beijerinck[57] identified a bacterium, named by him as *Azotobacter*, that occurs frequently in all marine habitats. Stoklasa[58] demonstrated that another soil bacterium, *Radiobacter*, reveals the same capability, and that "it is possible that a large number of similar bacteria will be found in soil"[59] — as many researchers anticipate.

Another series of bacteria that are also capable of assimilating nitrogen from the air are those which cause tumours and nodules to sprout on roots of the Fabaceae and other plants. Among these are *Bacterium (Rhizobium) radicola* and *B. (Rhizobium) beijerinckii* as well as another remarkable species that lives on the roots of *Datisca cannabina*[60].

Not only are bacteria able to assimilate gaseous nitrogen from the atmosphere, but also from the soil as ammonia or

[52] Of course, this sentence sounds rather paradoxical, since quite the opposite sentence, namely, that fungi are characterized by an inability to produce organic substances themselves and that they therefore need ready-made organic food to live, can be found everywhere, in all textbooks and the literature. For example, Zopf says in his monograph "Die Pilze" (p. 439) : "A priori all we know is that the fungi are not able to synthesize organic substances by themselves (because they lack chlorophyll), and moreover that they must obtain preformed organic substances from the envorinment. But this is only true for carbohydrates. Regarding proteins, fungi are able to synthesize them from nitrogen in the form of salts or in the form of the free gas. Even if some fungi require organic substrate or even protein containing substrate, this is just a secondary trait caused by parasitism and saprophytic growth. For example, it would be incorrect to state that the seed plants are unable to synthesize organic substances from inorganic substances alone, just because a few representatives have lost this ability due to parasitism; the same applies to fungi, which should not be seen as organisms "that are unable to generate organic compounds by themselves."

[53] I am not aware of any irrefutable facts that would demonstrate that in a plant cell lacking any form of plastids, either carbohydrates or proteins could be produced from inorganic substances alone.

[54] Mereschkowsky, C., Über Natur und Ursprung der Chromatophoren im Pflanzenreich. Biol. Centralbl. Vol. XXV, No. 18, 1905.

[55] It would be extremely important to clarify by means of detailed and complicated experiments whether the so-called "rot fungi" from the group of phycomycetes are able to assimilate nitrogen in the form of inorganic salts. On this question, a positive hint by Laurent exists regarding *Mucor racemosus*. (Laurent, E., Recherches sur la valeur comparée des nitrates et des sels amoniacaux comme aliment de la levure de bière et de quelques autres plantes. Annales d. l'Institut Pasteur Vol. 3, 1899, p. 362) and a rather negative hint by Falk with regard to *Sporodinia grandis* (Falk, R., Beiträge zur Biologie der Pflanzen Vol. 8, 1901, p. 213). Apart from the fact that the observations of both authors contradict each other, one has to keep in mind that Laurent calls *Oidium lactis* (ascomycete) "cette mucédinée" (l. c. p. 370). Accordingly, one can doubt whether he was really dealing with phycomycetes in this case.

[56] Winogradsky, S., Comptes Rendus d. l'Acad. d. Sc. Paris, Vol. CXVI, 1893. *ibid*: Vol. CXVIII, 1894 – Archiv des Sciences biologiques de l'Institut de Médecine Expérimentale. St. Pétersburg, 1895, Vol. III, Issue 4. – *Clostridium pasteurianum*, seine Morphologie und seine Eigenschaften als Buttersäureferment. Centralbl. f. Bakteriologie, Vol. 9, 1902, p. 3.

[57] Beijerinck, M., Centralbl. F. Bakteriologie (Series II), Vol. VII, 1901, p. 561. – The *Azotobacter* discovered by this author raised doubts for a while as to whether it was really capable of assimilating nitrogen from the air, but as shown by the experiments from A. Koch (see Lafar, Handbuch der technischen Mykologie, Vol. III, Jena 1904, p.9) there is no reason for these doubts. — Benecke and Reutner believe that *Azotobacter* is not a bacterium at all, but belongs to the cyanobacteria and consider it a colourless form of *Aphanocapsa*.

[58] Stocklasa, J., Über die chemischen Vorgänge bei der Assimilation des elementaren Stickstoffes durch *Azotobacter* und *Radiobacter*. Berichte d. deutsch. botan. Ges. 1906, Vol. 24, p. 22.

[59] Schmidt, Johs und Weis, Fr., Die Bakterien. Jena 1902, p. 115.

[60] Montemartini, L., Atti Acad. dei Lincei. Roma (5), Vol. XVI. 1906, p. 144, cited after Czapek, l. c.

nitrate salts. Such bacteria are numerous[61], and with respect to their requirement of nitrogen they are divided into obligate autotrophs and facultatively autotrophs. Among the obligate autotrophs are, in addition to the nitrogen assimilating bacteria known to us from the findings of Winogradsky, most probably also the sulfur bacteria *Beggiatoa, Thiotrix* etc., in addition the iron bacteria and probably also the purple bacteria.

[300] There are also bacteria known that assimilate carbon from CO_2 in order to synthesize organic molecules from this simple inorganic substance and water[62].

Czapek[63] writes: "Surprisingly many microbes are able to utilize the simplest compounds of carbon chemistry and in this regard are no longer distinct from nitrifying organisms with chemosynthetic carbonic acid assimilation". The CO_2 autotrophy of bacteria, that is, the ability to fix CO_2 was first demonstrated by Winogradsky[64] for nitrifying bacteria that do not require light energy for CO_2 assimilation, as they are able to assimilate CO_2 in the dark by using chemical energy that they obtain from the oxidation of ammonia to nitrite or the oxidation of nitrite into nitrate.

According to Kaserer[65], the soil bacterium *Bacillus pantothropus* reduces CO_2 to formaldehyde and subsequently to more complex compounds by oxidizing hydrogen. Another bacterium detected by Beijerinck and Delden[66], *Bacillus oligocarbophilus*, is reported by the same author to initially reduce CO_2 to CO, from which it then synthesizes its organic compounds without releasing oxygen. The same ability to assimilate CO_2 without producing oxygen was reported by Niklewski[67] for colorless bacteria. — The same is also true for marine sulfur bacteria (thiobacteria) according to Nathanson[68]. Beijerinck[69] confirmed the findings of Nathanson and showed that two freshwater bacteria living in the mud of ditches, *Thiobacillus thioparus* and *Th. denitrificans*, are able to fix CO_2 in the dark. [301] The energy required for this chemosynthesis comes from oxidizing sulfur. The former oxidizes carbon disulfide *[schwefeliger Kohlenstoff]* to sulfur or oxidizes $Na_2S_2O_2$ or $Na_2S_4O_6$ to Na_2SO_4 and S, respectively. The latter gains its energy from oxidizing sulfur and reducing nitrate (for lack of available free oxygen) into free nitrogen according to the reaction:

$$6KNO_3 + 5S + 2CaCO_3 = 3K_2SO_4 + 2CaSO_4 + 2CO_2 + 3N_2 .$$

If one provides these bacteria with sugar or other organic substances as carbon source they will always prefer CO_2 or inorganic salts of CO_2 for synthesis of their organic compounds.

In this way a continuous transformation of inorganic substances in the presence of sulfur or hydrogen sulfide into organic compounds takes place in the mud of ditches and ponds as well as in total darkness on the ocean floor.

It appears probable that the remaining sulfur bacteria, the iron bacteria and possibly the purple bacteria belong to the CO_2 autotrophs[70].

The Fungi.

Not a single animal can live from carbohydrates (sugar, starch) and fat alone without a supply of nitrogen containing substances because the amoeboplasma of animals is incapable of synthesizing nitrogen containing compounds like proteins from inorganic substances[71]. Fungi, however, which consist of mykoplasma, possess this ability and therefore most fungi require neither protein nor any other nitrogen containing organic compound as food.

Although fungi utilize organic compounds like carbohydrates as carbon source, Pfeffer[72] presumes that it may be possible that behind the apparent carbon heterotrophy in fungi sometimes a true autotrophy is hidden; presumably the fungi gain their energy to assimilate CO_2 from the oxydation of carbohydrates[73]. *[No fungi are autotrophic.]* [302] This is all the more plausible as there are numerous examples known among bacteria, from which the fungi evolved.

But with respect to nitrogen, fungi appear as autotrophic organisms in the same way as bacteria and cyanobacteria do. It is well known that they can live from substrates, that apart from carbohydrates, which serve as a source of bicarbonate *[die als Quelle der Kohlensäure erscheinen]*, consist solely of inorganic substances. Accordingly, fungi obtain nitrogen from nitrogen containing salts, with a preference for ammonium containing over nitrate containing salts, in contrast to plants[74].

But fungi are also able to assimilate free nitrogen from the air in the same way as bacteria[75]. Of this there can be almost

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[61] Lafar, Fr., Handbuch der technischen Mykologie. Vol. I, Jena 1904, p. 412.

[62] Lafar, l. c., p. 410.

[63] Czapek, F., Die Ernährungsphysiologie der Pflanzen seit 1896, Progressus rei botanicae, Vol. I, Issue 2, Jena 1907, p. 479.

[64] Winogradsky, S., l. c.

[65] Kaserer, H., Die Oxydation des Wasserstoffes durch Mikroorganismen. Centralbl. f. Bakteriol. (II. Department), Vol. XVI, 1906, p. 681.

[66] von Delden, A., Centralbl. f. Bakteriol. (Series II), Vol. II, 1903, p. 81.

[67] Niklewsky, M., Ein Beitrag zur Kenntnis wasserstoffoxydierender Mikroorganismen. Bulletin d'Acad. d. Cracovie. Classe des sc. mathem. et nat. 1906, p. 911.

[68] Nathansohn, Über eine neue Gruppe von Schwefelbakterien und ihren Stoffwechsel. Mitteil. a. d. zoolog. Station zu Nepal, Vol. 15, 1902, p. 655.

[69] Beijerinck, M., Phénomènes de reduction produits par les microbes. Archives Néerland. des sc. ex. et natur. Sér. II, Vol. IX, 1904, p.131. – Referat im Botan. Centralblatte, 1904, p. 298. – See also Centralblatt f. Bakter. (Series II), Vol. XI, 1904, p. 693.

[70] Lafar, Fr., Handbuch der technischen Mykologie. Vol. I, Jena 1904, p. 418. This ability regarding the purple bacteria is based on their production of oxygen under the influence of light (W. Engelmann's method), about which Molisch has recently (Molisch, H., Die Purpurbakterien nach neuen Untersuchungen, Jena 1907) — raised strong doubts. However, it permits the assimilation of CO_2, but without producing oxygen, as some other bacteria do (see above).

[71] It would be extremely important to examine this rate in relation to lower animals. – Nobody has tried to feed a *Hydra*, for example, with salts and organic, but nitrogen-free substances. It is unknown to me whether similar experiments were made with infusoria.

[72] Pfeffer, W., Pflanzenphysiologie, Vol. I, 2. Edition, 1904.

[73] It would be extremely important to demonstrate by direct experiments the possible existence of autotrophy in relation to carbonic acid in fungi.

[74] Lafar, Fr., Handbuch der technischen Mykologie. Vol. I, Jena 1904, p. 402.

[75] The view of Frank and some others that green plants can assimilate nitrogen from the atmosphere can now be regarded as refuted on the basis of a whole series of investigations. The results of Boussingault's classical experiments, which first proved the inability of plants to assimilate free nitrogen, thus stand. For what Frank and others attributed to the ability of green plants, was in fact carried out by the soil bacteria. For the literature on this subject see:

no doubt if one considers the parasitic fungi of the mycorrhiza according to experiments carried out by Nobbe and Hiltner using *Podocarpus*, a fungus that exhibits excellent growth in pure quartz sand lacking nitrogen altogether, or the observations of P. E. Müller on pine mycorrhiza[76]. *[No fungi are diazotrophic.]*

Especially interesting in this respect are experiments carried out by Ternetz[77] using a fungus that grows on the roots of various Ericaceae. Ternetz cultivated this fungus axenically in a nitrogen deficient medium. In this medium the fungus grew rapidly, and Ternetz, through exact analyses, was able to demonstrate the increase in nitrogen content, which only could have its source in the atmospheric nitrogen. [303] Ternetz had no doubt that she was dealing with a true fungus, as demonstrated by the mycelium, which was divided by septae and developed fungus specific propagation organs, pyknidia.

There can be no doubt at all that the mycorrhiza contains true fungal hyphae belonging to the Hymenomycetes and Nectariaceae. It is just as certain, at least with respect to the endotrophic mycorrhiza, that these fungi assimilate free nitrogen from the air to synthesize their own protein[78]. *[probably contaminating N_2 fixing bacteria, perhaps actinomycetes like Frankia in the case of the mycorrhiza.]*

[end Part II, vol. 30, No. 9, May 1, p. 303; begin Part III, vol. 30, No. 10, May 15, page 321]

(Conclusion).

The Cyanobacteria.

Though to my knowledge experiments to demonstrate carbon assimilation in cyanobacteria have not been carried out to date[79], the presence of chlorophyll and the ability to release oxygen under illumination as may easily be shown using the bacterial method, is evidence enough that also cyanobacteria are autotrophic with respect to the assimilation of carbon.

Are cyanobacteria autotrophic with respect to nitrogen assimilation as well?

There are many reasons to believe that they can live without preformed proteins and that they synthesize their own proteins from inorganic compounds. This is indicated by the fact that they often multiply to immense numbers in the open ocean, thereby causing red or yellow tides. [322] It seems extremely unlikely that the open ocean can contain such large quantities of nitrogen containing organic compounds *[to support that growth]*. There are experiments carried out by Loew[80] with *Nostoc* demonstrating that this cyanobacterium is able to assimilate inorganic salts as a nitrogen source as it grows well under 0.1% KNO_3.

Yet there are also reasons to believe that cyanobacteria, similar to bacteria and fungi, may assimilate free nitrogen from the air[81]. Indicative of this are cyanobacteria living in the roots of cycads, where they form coral-like protuberances. Such growths can occur in large numbers and often break through the soil's surface. Gardeners carefully avoid to damage them because they consider it dangerous for the plant, based on the assumption that the roots breathe through such structures. Of course, this explanation is not correct, but the benefit for the plant appears to be evident. Koch[82] surmises that "it is not a mistake to assume that they are related to nitrogen supply for the plant", that is, that their role is analogous to that of the fungi of mycorrhiza in fixing free nitrogen.

Plastids.

It is known that plastids possess the ability to assimilate CO_2 and to build up complex organic compounds from this gas and water.

It is less well known as to whether plastids are able to synthesize more complex molecules like proteins from inorganic substances. [323] There are equivocal hints that the synthesis of proteins occurs just inside the plastids: where they are most frequent — as in leaves — we also find the majority of proteins. On the other hand, we observe that nitrate, which is required to synthesize proteins and which can be found elsewhere in the plant, disappears in the leaves where it must be assimilated into protein. The amount of proteins in leaves increases simultaneously. Finally, as Sachs[83] demonstrated, proteins emerge from the leaves in which primarily the amino acids as precursors of proteins accumulate. From this we can conclude that leaves are the site of protein synthesis. But inside the leaves there is also the majority of chlorophyll, the plastids are also mainly concentrated in the cells of leaves. If proteins are also formed in the plant roots and apparently only from

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Koch, A., Der Kreislauf des Stickstoffes, in Lafar, Handbuch der technischen Mykologie, Vol. III, Jena 1904, p. 12 ff.

[76] However, it must be kept in mind that Müller recently (Berichte d. deutsch. botan. Gesellsch. 1906, Vol. 24, p. 230) cites experiments according to which the mycorrhizal fungi of pine trees are apparently unable to assimilate nitrogen from the air.

[77] Ternetz, Ch., Assimilation des atmosphärischen Stickstoffes durch einen torfbewohnenden Pilz. Berichte d. deutsch. botan. Gesellsch. 1904, Vol. 22, p. 267.

[78] There has recently been a complete disagreement about the ability of molds (*Aspergillus*, *Penicillium*) to assimilate the free oxygen in the air. Some (Saida) think that *Mucor* also has this ability, but this seems very unlikely considering that *Mucor* is not a fungus.

[79] Kohl, F., Über die Organisation und Physiologie der Cyanophyceenzelle. 1903.

[80] Loew, O., Verhalten niederer Pilze gegen anorganische Stickstoffverbindungen. Biol. Centralbl., Vol. X, 1890, p. 591. *[footnote missing, belongs at that spot]*

[81] See the experiments of Bouilhac and Giustiniani (L'année biologique, 1903, p. 204) which prove that *Nostoc* and *Anabaena* can develop vigorously in a medium that is completely nitrogen-free; presumably they draw the nitrogen they need from the air. Unfortunately, this cyanobacterial culture was not free of bacteria and therefore it is possible that the assimilation of nitrogen was not only carried out by the cyanobacteria but also by the bacteria, or even only by the latter. Beijerinck provided substantial evidence, according to which *Nostoc* and *Anabaena*, two cyanobacteria, are able to fix atmospheric nitrogen (Beijerinck, Centralbl. f. Bakteriol. Vol. VII, 1901, p. 562). But Czapek (Biochem. d. Pflanzen, Vol. II, p. 230) also counts these experiments, which were not supported by the necessary analyses, as insufficiently convincing.

[82] Koch, A., Der Kreislauf des Stickstoffes, in Lafar, Handb. d. techn. Mykologie, Vol. III, Jena 1904, p. 64.

[83] Sachs, J., Vorlesungen über Pflanzenphysiologie, Leipzig 1882. – See also Pfeffer, W., Pflanzenphysiologie, Leipzig, Vol I, 1897, p. 402, and Czapek, F., Biochem. d. Pflanzen, Vol. II, Jena 1905, p. 211.

amides, the roots also have plastids[84]. Taking all such data into consideration, we have good reasons to pose the question "are plastids capable of synthesizing proteins?" — and to answer it in a positive sense.

This above question as to whether plastids are the source of protein synthesis may also be asked in the following and more correct manner:

Who is responsible inside the plant cell for the synthesis of proteins? Is it the plastids (mykoplasma) or is it the cytoplasm (amoeboplasma) of the cell? As we will see, it is by no means a difficult task to obtain a concise answer to this question. We only would have to observe whether plant cells from which we remove the plastids would be able to continue with protein synthesis or not. In case the plant cell following this surgery would not be able to continue synthesizing proteins from salts and gases, then this capability would only come from the plastids.

But how would it be possible to perform such a delicate surgery of removing the plastids from the living cell without damaging it? Is it even possible to perform such an experiment?

It becomes evident that such an experiment is possible. Such an experiment has already been conducted, and with the inimitable artistic skill of the greatest of all artists, whose name is nature.

[324] There are some diatoms belonging to the genus *Nitzschia* like *N. leucosigma* Benecke and *N. putrida* Benecke[85], which, as a consequence of living in muddy water enriched with organic substances, have lost their pigmented plastids *[Endochrom, endogenous pigment]* and have lost them so fundamentally that it has become impossible to restore them by any means. The loss of pigmented plastids can be achieved in many other diatoms as well if one cultivates them in media enriched with organic substances. However, as Karsten[86] has shown, there remain unpigmented remnants of plastids either as colorless platelets or as pigmented but very small bodies. These reduced plastids recover to their original size and form, however, if one transfers the diatoms into media containing only inorganic compounds. But this does not happen in the case *Nitzschia putrida*. In this species, the influence of organic food, that is, the influence of heterotrophic nutrition, has continued for so long that the initially pigmented diatoms forever changed into an unpigmented species lacking any trace of plastids. The plastids cannot be restored under any conditions.

Nature has performed an experiment that we are not able to perform ourselves: the plastids were removed so carefully that the cell itself was not harmed at all.

All that remains is to cultivate this diatom with its unharmed, normal amoeboplasma, which no longer contains any plastids as mykoplasma, in a medium that only contains inorganic substances in order to see whether the cell is now capable of synthesizing proteins from inorganic compounds, that is, whether it can live and multiply under such conditions or not.

This experiment has been done by Karsten, although that author was not engaged with the question that is currently of interest to us. And what were the results? "In pure ocean water the cells died regularly after 24 hours. In contrast, they behaved quite well and showed more or less vigorous movement even in low concentrated media containing glucose, asparagine, glycine, peptone, glycerol both in dark and under light[87]."

[325] From these experiments, whose importance Karsten apparently failed to notice, it becomes evident which cellular part of the diatom fulfills the synthesis of proteins from inorganic substances: this function is performed exclusively to the plastids[88]. The amoeboplasma of the diatom cell is not able to fulfill this task; it may starve from hunger as far as one does not supply it with organic substances[89].

From this we may set up the following equation:

Diatoms – Plastids = Animals

and from that

Animals + Plastids = Plants.

We begin to recognize that across the entire spectrum of the mykoid kingdom, including free living members — bacteria, fungi, cyanobacteria — also among representatives that live symbiotically (plastids), we are confronted with numerous examples of autotrophic nutrition, that is, the ability of the mykoid plasma to synthesize complex organic molecules from simple inorganic ones. And if, at the same time, we do not see one single similar example among the organisms that are made of the amoeboplasma, we may conclude with certainty that both plasma lineages, the mykoplasma and the amoeboplasma, must be fundamentally different from one another, that the kingdom of the mykoids is made from a plasma totally different from that of the animal and plant kingdom.

V. Movement.

The amoeboplasma possesses the ability to perform active movement, either in the form of amoeboid shape modifications or as muscle contractions; [326] the amoeboplasma also

[84] If proteins are also formed in the plant roots and apparently only from amides, the roots also have plastids.

[85] Benecke, W., Über farblose Diatomeen der Kieler Föhrde. Pringsheim's Jahrb. f. wissensch. Botanik, Vol. 35, 1900, p. 536.

[86] Karsten, G., Über farblose Diatomeen. Flora oder allgem. botan. Ztg., Vol. 89, 1901, p. 404.

[87] Karsten, G., Über farblose Diatomeen. Flora oder allgem. botan. Ztg., Vol. 89, 1901, p. 426.

[88] This is just as clear and unquestionable as the following attempt would be: Suppose we have an illuminated room in which a lamp is burning on the table; if we carry the lamp out of the room and the room is completely shrouded in darkness, and if we repeat this attempt several times with the same result, we have of course the right to assert that the light in the room comes from the lamp. This conclusion will definitely be correct and exactly in to the same degree correct and unassailable as the conclusion from Karsten's experiments showing that the assimilation of protein in diatoms is performed by the plastids and only by the plastids. But if the plastids play such a role in the diatoms, they must of course play the same role in all other plants. — In this way, we now have direct proof that the synthesis of protein in plants takes place in the plastids.

[89] Unfortunately, Karsten did not attempt to cultivate *Nitzschia putrida* in a solution containing inorganic salts and some hydrocarbon *[sic, Kohlenwasserstoff]*, for example sugar. Then a second question would be solved: Can a diatom that has lost its pigmentation live like a fungus, that is, synthesize its protein from inorganic substances, if provided a source of organic carbon. It would be extremely interesting to conduct such an experiment.

frequently creates pulsating vacuoles. The mykoplasma is totally incapable of moving like an amoeba and never creates pulsating vacuoles.

We do not consider animals further in this context as their ability to move has always served as the main character to discriminate them from plants. — But also among plants movements are more common than generally recognized and the plasma of a plant cell moves just like an amoeba or a rhizopod. The amoeboid movement of the plasma, for instance, can be observed in diatoms, responsible for changing the position of the alga. Furthermore, it can be observed in green algae of the Siphonales and Siphonocladales; for instance, in the macrosopic multinuclear alga *Caulerpa* the interior is traversed by protoplasmic strands in which the protoplasma visibly streams. It is very easy to also observe protoplasmic streaming in the phycomycetes which, as is now generally accepted, are not fungi but algae that have lost their pigmentation. It is particularly easy to observe streaming in *Saprolegnia*. In another phycomycete, *Monoblepharis*, the spermatozoids exhibit amoeboid-like movements in that they crawl upon the oogonia like little amoebae. In the green alga *Draparnaldia*, the gametes initially possess flagella, but they soon discard them and their further contact and fertilization is maintained by amoeboid movements. In the Characeae the circular movement of the plasma is one of the most exciting phenomena that one can encounter under the microscope. But also among flowering plants, movements of the protoplasma are found, circular movements as in *Valisneria spiralis* and *Hydrocharis*, or streaming movements as in the staminal hairs of *Tradescantia virginiana*, *Lamium*, pumpkin etc. are widespread[90)].

One has to keep in mind that the movement of the protoplasma in plant cells is of two types: [327] p r i m a r y or continuous if the streaming is continuously observed in undamaged cells and s e c o n d a r y movement, which occurs only under the influence of external effects, for instance following the preparation of sections or under the influence of strong changes in air and temperature conditions. Even if one takes into account the streaming of the irregularly agitated cytoplasm, which under normal condition is at rest, the number of cases of amoeboid movements of the plant cytoplasm is enormous[91)].

Besides the amoeboid movement and the muscle contractions which may be deduced from the former ones, the amoeboplasma exhibits another remarkable form of movement that is manifest in contractile vacuoles. Cases where such vacuoles exist in lower animals are widely known. But also in lower plants they are widespread, namely in the mobile stages, in zoospores and gametes. In higher animals and plants the contractile vacuoles disappear; in animals because various complex organs become responsible for excretion of waste material, in plants because there exists a cellulose layer outside each cell closely wrapping the cytoplasm that renders the function of similar organs impossible.

Let us now consider the situation within the kingdom of the mykoids.

The fungi possess a completely immobile cytoplasm, with no traces of amoeboid-like movements or contractile vacuoles ever being observed. If any movements whatsoever have been observed inside the hyphae of true fungi, they do not reflect active movements of the amoeboid plasma, as work by T e r n e t z [92)] has rendered likely, but instead appear to reflect passive movements caused by the turgor of the cells. Therefore its character is quite different from that of the amoeboid movements in plant cells in that the entire mass of the protoplasma shifts into one direction or the other, similar to low tide and high tide[93)].

[328] The cyanobacteria likewise do not exhibit any movements of their plasma[94)], the same applies for plastids[95)]. Neither have contractile vacuoles.

With respect to the bacteria they also show no amoeboid agitation, and are also completely lacking contractile vacuoles.

Many bacteria move as a whole, however, with the support of their flagella. At first glance these movements do not differ from those of zoospores, infusoria, or gametes. Yet closer

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[90] See with regard to this question W i g a n d , Botan. Hefte, Forsch. a. d. botan. Garten zu Marburg. 1. Issue, 1885, where all known cases of movement of plasma in plant cells are compiled and classified. The view expressed by K e l l e r that all movements of the plasma in plant cells are secondary movements, that is, are caused by tissue rupture and injury, appears undoubtedly to be exaggerated and one-sided. Incidentally, this question has no relevance for our purpose. What is important for us is to know whether the plasma has any amoeboid movement at all, of whatever kind, primary or secondary.

[91] H a u p t f l e i s c h states: The flow of plasma is therefore present in all tissue forms, it is not absent in any of them (H a u p t f l e i s c h , P., Untersuchungen über die Strömung des Protoplasmas in behäuteten Zellen. Pringsh. Jahrb. f. wissensch. Botan., Vol. XXIV, 1892, p. 185). *[footnote 91 missing in the text, probably belongs at the position indicated]*

[92] T e r n e t z , Ch., Protoplasmabewegung und Fruchtkörperbildung bei *Ascophanus corneus* P e r s . P r i n g s h . Jahrb. f. wissensch. Botan., Vol. XXXV, 1900, p. 273. W o r o n i n observed similar movements in another ascomycete (*Ascobolus pulcherrimus*) (W o r o n i n , M., Beiträge zur Morphologie und Physiologie der Pilze, II. Series). A r t h u r observed similar movements of passive character in *Rhizopus nigricans* (A r t h u r , J., Annals of Botany, Vol. XI, 1897).

[93] With regard to the fact that in the literature one sometimes comes across detailed descriptions of the amoeboid movements in mushrooms, based on misunderstandings, it is appropriate to recall H o f f m a n n . "The plasma of the spores and of the germination tube is contractile and motile like that of animal sarcode *[sarcode is an archaic term for animal protoplasm]*. Neither is immediately visible because the movement is much slower than that of the minute hand on a clock. But after a few hours one observes that the plasma, moving forward as the tube extends, leaves, as a whole, the parts of the tube that it had previously occupied (for example *Agaricus oreades*). The movement is to be described as streaming. " (H o f f m a n n , H., Untersuchungen über die Keimung der Pilzsporen. P r i n g s h . Jahrb. f. wissenschaftl. Botan., Vol. II. 1860, p. 318). From this description it becomes clear that in this given case we are dealing with growth, but not with amoeboid movement. The plasma of the fungi grows but does not move "like animal protoplasm".

[94] In some filamentous cyanobacteria, e.g. *Oscillaria*, *Beggiatoa* and *Spirochaete* (I do not consider the latter two forms as bacteria, although they are colourless; they are cyanobacteria that have lost their pigmentation), one notices a movement of the whole filament, one forward and one backward, which seems to be caused by the production of mucus on the surface of the filaments; in addition, a snake-like movement is observed, the cause of which remains completely unknown.

[95] The change of shape in plastids is very significant and sometimes, as in the case of the division of diatoms, it happens relatively quickly, but here too we are dealing with a growth phenomenon or division, but not with real amoeboid movement, since the change of contours is extremely slow and very passive. In my opinion, S e n n ' s observations do not contradict this sentence.

observation reveals essential differences among bacterial flagella and those of amoeboids.

The flagella of the amoeboids may be considered as modified filipodia, that is, thin and filamentous pseudopodia of rhizopodia, heliozoa or radiolaria[96]. As with the majority of filipodia and with all typical flagella of the ciliated epithelium there exists a strong central axis extending into the interior of the protoplasmic body of the cell, [329] either ending in the nucleus or in any strong and intensely staining body. Belajeff[97] has proven that the flagella of the water fern spermatozoids terminate at densely staining bodies which Webber initially named blepharoplasts and which according to Belajeff may be derived from centrosomes. Ikeno[98] confirmed this view by demonstrating it for cycads and, more recently, for liverworts (*Marchantia*). During spermatogenesis in *Marchantia*, the centrosome persists following disintegration of the spindle apparatus and becomes the basis of the flagella. The same was reported with great distinctness for the zoospores of the myxomycetes by Plenge and E. Jahn[99]. During division of the nucleus at zoospore formation centrosomes become visible at the tip of the spindle, and following completion of the cell division each of the two centrosomes releases one flagellum which remains connected with the nucleus via the corresponding half of the spindle.

If one recalls that the axis of the spermatozoid flagella of various animals (human, rat, salamander, butterfly, *Helix*) originate from the centrosome (more correctly the centriole)[100], that furthermore the axis of the pseudopodia in the protozoans *Acanthocystis*, *Raphidiophris*, and *Actinolophus* originate from the intensely staining nucleus, that in *Camptonema nutans* each pseudopodium which slowly moves like a flagellum ends inside the cell at a specific structure[101], and finally that the epithelial flagella of all animals including vertebrates end inside the cell at a specific body[102] like in infusoria, [330] it would hardly be wrong to say that such a constructional feature appears as a general rule, that is, that the flagella of the amoeboids are in close contact with the centrosome. In every case one may claim that the basis of flagella is connected with the so-called basal body which most probably originates from the centrosome[103].

There is nothing similar in bacteria where the flagella directly extrude from the outer envelope of the cell. Instead, according to Fischer[104], one observes a peculiar phenomenon: if one separates the envelope from the cell body following plasmolysis, the flagella adhering to the outer side of the cell wall continue to move as normal, thereby also setting the bacterium into motion. Nothing similar can be observed in the amoeboids, i.e. plants and animals.

Even if we disregard the differences between the flagellar movement of the amoeboids and mykoids, the principle itself, which is responsible for the movement, appears to be completely different in the two cases. The facts presented in this chapter convincingly show that not a single member of the mykoid kingdom exhibits traces of amoeboid movement. Nor does a single member possess contractile vacuoles. The amoeboid plasma is highly mobile, the mykoid plasma is immobile. That once again indicates that a deep and fundamental difference must exist concerning the structure of the amoeboplasma and the mykoplasma.

VI. Chemical composition.

A remarkable difference between the mykoplasma and the amoeboplasma is also seen in their chemical composition. [331] — In this respect, however, we are faced with considerable problems caused by the lack of sufficient data to support this statement. The reason is that to date no one has focused on the existence of two kinds of cytoplasm. Therefore, it is not surprising that specific observations providing putative answers to questions under interest in this respect were made occasionally while investigating quite divergent topics. As Reinke[105] correctly states: "If a problem has not been recognized, it cannot be subjected to investigation." — This statement highlights the significance of all scientific hypotheses and theories, even those that have failed — as the most important stimuli of scientific progress.

Nonetheless, despite scanty observations, we are able to a certain extent, to ascertain, in a fairly plain manner, though not with full clarity, essential differences in the chemical composition of the two plasma lineages. Apparently the mykoplasma appears to be enriched in phosphorus compared to that of the amoeboplasma. Hints come from facts obtained through the analyses of ashes of both animals and plants, which in great number are compiled and published in Wolff's "Analyses of Ashes"[106].

Let us consider especially the data from fungi. From these we see that the P_2O_5 content is highly related to that of plants.

[96] Gurwitsch, A., Morphologie und Biologie der Zelle. Jena 1904, p. 38 ff.

[97] Belajeff, W., Über die Centrosome in den spermatogenen Zellen. Ber. d. deutsch. botan. Gesellsch., Vol. 17, 1899.

[98] Ikeno, S., Die Spermatogenese von *Marchantia polymorpha*. Beihefte zum botan. Centralbl., Vol. XV, 1903. See also: Die Blepharoplasten im Planzenreich. Biolog. Centalbl., Vol. XXIV, 1905. – The presence of centrosomes in liver mosses has been denied by various observers (Miyake, Escoyez and others), but since v. Leeuven-Reijnwann (v. Leeuven-Reijnwann, W. et J., Über die Spermtogenese der Moose. Ber. d. deutsch. botan. Gesellsch., Vol. XXVI-a, 1908, p. 301) has recently reconfirmed their presence in *Fegatella*, *Pellia* and *Mnium* with a clarity that leaves nothing to be desired, one has no reason to doubt this fact.

[99] Jahn, E., Myxomycetenstudien. Ber. d. deutsch. botan. Gesellsch., Vol. 22, 1904, p. 84.

[100] Häcker, V., Praxis und Theorie der Zellen und Befruchtungslehre. Jena 1899.

[101] Gurwitsch, A., Morphologie und Biologie der Zelle. Jena 1904, p. 45.

[102] Gurwitsch, A., l. c., p. 64, Fig. 30, p. 93, Fig. 43.

[103] There are quite a few very well-founded indications that the basal bodies originate from the centrosome, although work has recently been published which apparently proves that this body originated independently. Thus Wallengren demonstrates it in relation to the ciliated epithelia of the Lammellibranchiata (Wallengren, H., Zur Kenntnis der Flimmerzellen, Zeitschr. f. allgem. Physiologie, Vol. V, 1905, p. 357). But in the given case, considering the extreme small size of centriols and their inconsistency with regard to their stainability, the positive indications carry more weight than do the negative ones.

[104] Fischer, A., Vorlesung über Bakterien. Jena 1903.

[105] Reinke, J., Ber. d. deutsch. botan. Gesellsch. 1904, p. 100.

[106] Wolff, E., Aschenanalysen von landwirtschaftlichen Produkten, Vol. I, 1871. — Vol. II, 1880. – See also König, Chemie der menschlichen Nahrungs- und Genussmittel. 3. Edition, 1889. — Liebig, J., Chemie in ihrer Anwendung auf Landwirtschaft und Pflanzenphysiologie. St. Petersburg, 7. Edition, 1864 (Russian).

If one compares the percentages of phosphoric acid in the ash of plants, starting with algae and ending with higher plants, with the corresponding data from fungi, the differences are striking:

[331–332]

Plants.		Fungi[107].	
Fucus vesiculosus (8) [108]	2.89	Sphacelia segetum	15.44
" serratus (3)	2.96	Dito on rye	58.66
" nodosus	1.67	" " "	53.88
Laminaria digitata (6)	2.91	" " barley	43.60
Laminaria saccharina (3)	3.72	" " smooth brome	40.47
Sargassum vulgare (3)	1.84	Cryptococcus fermentum	53.84
Polysiphonia elongata	1.76	Dito, bottom yeast	59.38
Delesseria sanguinea (2)	2.40	" wheat beer yeast	54.74
Ceramium rubrum	2.95	Tuber cibarium	32.96
Enteromorpha intestinalis	2.18	Helvella esculenta	39.10
Ulva latissima	1.61	Morchella esculenta	39.03
Algae in general (23)	2.85	" conica	37.18
Sphagnum cuspidatum	3.00	Agaricus campestris[109]	15.43
Forest moss	6.11	Boletus, birch polypore[109]	18.61
Hypnum schroeberi	12.38	Yeast	44.76
" splendens	20.21	"	58.87
" triquetrum	13.51	"	53.44
Sphagnum species	9.31	"	55.63
" near Berlin	6.33	Saccharomyces mycoderma	54.53
Aspidium felix femina	3.32	" cerevisiae	54.74
" " mas	2.56	Boletus edulis	25.06
" " leaves	15.60	" annulatus	21.74
Asplenium trichomanes	10.13	" aurantiacus	20.27
Osmunda spicant	1.76	Claviceps purpureus	45.12
Pteris aquilina	5.15	Agaricus cantharellus	31.32
Male fern (9)	7.58	Clavaria flava	35.07
Lycopodium (6)	5.77	Sclerotinia libertiana[110]	48.67
Fir, branches and needles	8.72	Mutterkorn[110]	45.00
Spruce needles (8)	16.00	Chanterelle[110]	31.32
Oat (38)	7.17	Truffle	54.21
Hay (106)	7.11	Morchella esculenta	37.75
Grasses (107)	7.37	Tuber cibarium	30.85
Clover flowers (113)	9.63	Boletus edulis	20.12
Turnip (149)	12.18	Edible fungi (mean from 9 observations)	33.71
Tobacco leaves (63)	4.66	Mould spores[110]	39.64
Spinach (2)	10.25		

According to Zopf[111] the ash of fungi contains on average 40% phosphoric acid, unknown from any group of organisms belonging to the amoeboids. Fischer[112] states "Usually 50% or more account for the phosphoric acid of the entire ash which therefore reacts acidically". Bacteria are rich in phosphorus in the same way. "The large amount of phosphoric acid found in the ash of bacteria is striking", [333] as Schmidt and Weis[113] note. H. Fischer points out the "enormously high content of phosphoric acid in the ash of most fungi and bacteria"[114]. According to the calculations of Koppes[115] the content of phosphoric acid in the ash of *Bacillus prodigiosus* and *B. xerosis* accounts for 38.01 and 34.45%, respectively. For bacteria causing tuberculosis Schweinitz and Dorset found 55.23%, in later work they found 55.54 – 73.94% phosphoric acid in the ash of these bacteria.

The importance of those numbers is weakened at first glance because in some cases a high amount of phosphoric acid may be observed in the ash of plants, in certain cases not much less than in fungi[116]. — These apparent differences do not in fact exist. In all cases the high percentage of phosphorus is observed exclusively in seeds or in such parts of the plant containing seeds (as in flowers) or eventually in such parts of the plant rich in reserve substances (bulbs, tubers). One may be easily convinced that in all such cases the enriched amount of phosphorus is not due to the specific ingredients of the plant protoplasma, but traces back to the presence of substances either of proteinaceous or other nature which are laid down as reserve substances. This phosphorus is definitely not part of the

[107] Wolff, l. c., Vol. I, p. 134 and Vol. II, p. 110. – It is interesting to note that lichens, which consist of mykoids (fungi) and amoeboids (algae), already have a much lower phosphorus concentration (Wolff, l. c., p. 135).

[108] The numbers on the right (in brackets) indicate the number of analyses, although I have combined the individual data from Wolff and taken the means from all the cases listed by him.

[109] These two cases of low phosphorus concentrations, as well as some others, are explained by the unusually high potassium and partly sodium concentration.

[110] These data are taken from Lafar, Handbuch der technischen Morphologie, Vol. I, Jena 1904, p. 225. *[Mutterkorn, sclerotium of Claviceps purpurea, misspelled in the table as Mutterhorn, was intended.]*

[111] Zopf, W., Die Pilze, p. 388.

[112] Fischer, H., Die chemischen Bestandteile der Schizomyceten und der Eumyceten, in Lafar, Handbuch der technischen Mykologie, Vol. I, Jena 1904, p. 225.

[113] Schmidt, Johs and Weis, Fr., Die Bakterien, Jena 1902, p. 107.

[114] Fischer, H., l. c., p. 224.

[115] l. c., p. 225.

[116] Wolff, E. Aschenanalysen. Part I, 1871 — Part II 1880, Berlin, at various places.

protoplasma of a given organism, and the structures containing phosphorus appear like exotic bodies (e.g. as protein crystals). Such bodies, rich in phosphorus, mostly belong to the group of phosphoglobulines according to Cohnheim[117], which in the animal kingdom are caseins, in the plant kingdom phytoglobulines[118].

More problematic is the high amount of phosphorus in the ash of muscle, as shown in the following table for which I used the data published by Champion and Pellet[119], [334] which provide the percentage of P_2O_3 in the ash of muscles or entire animals, respectively.

	P_2O_3		P_2O_3
Human, muscles	37.5	Eggs	36.8
Chicken	36.5	Ox	39.5
"	36.3	Calf	39.9
Eggs	38.0	Sea fish	34.5

Regrettably, an analysis of the ash of cyanobacteria, as far as I know, has not been performed to date. Undoubtedly these mykoids will also possess a percentage of phosphorus not even less than that of fungi and bacteria[119b].

The nuclei, however, which according to my theory primarily consist of mykoplasma[120], are rich in phosphorus as is longstanding known: where there are many nuclei as for instance in young tissue or in sperms, there is much phosphorus.

But the nuclei allow us to step further into explaining the chemical differences of the two plasmas — the mykoplasma and the amoeboplasma. We saw above that the mykoplasma is generally enriched in phosphorus, the nucleus now allows us to determine the site where it is concentrated, that is, which chemical bodies contain it.

It becomes apparent that the abundance of phosphorus within the nucleus is caused by the presence of nucleoproteins, which are totally absent for the amoeboplasma (cytoplasm), apart from the chromidia *[chromatin bodies]* which, of course, come from the nuclei as Digby[121] has shown.

[335] This allows us to more strictly separate the two types of plasma with respect to their chemistry compared to what we previously did on the basis of high amounts of phosphorus, which are also present in seeds and muscles.

Now we can postulate that the mykoplasma (cell nuclei, cyanobacteria, bacteria) is rich in nucleoprotein, while the amoeboplasma (cytoplasm) possesses none.

Let us first demonstrate the presence of nucleoproteins and nuclein in mykoid organisms.

The macrochemical presence of nuclein in bacteria was first demonstrated in 1884[122] for *Bacillus subtilis* and *B. anthracis*. Later on, either true nucleoproteins or nucleic acids and hypoxanthine bases such like xanthine, guanine, adenine, which prove the existence of nucleoproteins, were found.

Substances showing characters of proteins have also been found by Iwanoff[123] in bacteria and fungi.

	Nitrogen	Phosphorus	Sulphur
Bacillus megaterium	16.32	1.85	2.10
" anthracis	16.00—16.27	2.16—2.25	1.95
Aspergillus niger I	15.66—15.74	0.84	1.12—1.21
" " II	15.19	0.99	1.23
Boletus edulis (cap)	15.64—15.84	1.08	2.14
Claviceps purpurea (sclerotia)	16.02—16.23	0.75	1.77

Most intensely investigated in this respect, however, are yeasts. Hoppe-Seyler identified in yeast the same nuclein which previously was detected in pus cells by Miescher, and Rossel succeeded to isolate considerable amounts of pure nuclein (nucleic acids).

[117] Cohnheim, O., Chemie der Eiweißkörper, 2. Edition, Braunschweig 1904.

[118] This subheading also includes other substances, for which see Czapek, F., Biochemie der Pflanzen, Jena, Vol. II, 1905, p. 742.

[119] Champion and Pellet, De la substitution equivalente des matières qui entrent dans la composition des végétaux et des animaux. Comptes Rend. d. l'Acad. d . Sc. Paris, Vol. 83, 1876, p. 488. – See also Katz, J., Die mineralischen Bestandteile des Muskelfleisches. Pflüg . Arch. f. Physiol., Vol. 63, 1896, p. 84, in which however the percentage of phosphorus is presented not in relation to the ashes but in relation to 100 parts of dry meat. — See in particular Wolff, E. Aschenanalysen, Part II.

[119b] Recently, Stoklasa, Brdlik and Ernest have convincingly demonstrated that chlorophyll also contains a fairly large amount of phosphorus (Stocklasa, J., Brdlik, W. and Ernest, A., Zur Frage des Phosphorgehaltes des Chlorophylls. Ber. d. deutsch. botan. Gesellsch., Vol. XXVII, 1909, p. 10). The negation of this fact by Willstätter is apparently a mistake.

[120] A discussion of this matter will appear in a subsequent article devoted to the question of which observations indicate that the composition of cell nuclei consists mainly of mykoplasma.

[121] Digby, L., Oberservations on "Chromatin bodies" and their relation to the nucleolus in *Galtonia candicans*, Annals of Botany, Vol. XXIII, 1909, p. 491.

[122] Fischer, H., Die chemischen Bestandteile der Schizomyceten und der Eumyceten, in Lafar, Handb. d. techn. Mykologie, Vol. I, 1904, p. 245, where the literature on this subject is also compiled.

[123] Iwanoff, K.S., Hofmeister's Beiträge z. chem. Physiol. u. Pharmakol., Vol. I, 1902, p. 524.

The quantitative determination of nuclein appears to be especially striking which was undertaken by Stutzer[124] using yeast and an undetermined mould which demonstrated the unusual high nuclein content inside the cells of these mykoids. The content of nitrogen containing substances of these species is as follows:

[336]

	Amides und peptones	Albumin	Nuclein
In brewer's yeast	10.11%	63.80%	26.09%
In mould	19.86%	39.39%	40.75%

Because in yeast and moulds the cell nuclei contribute only to a minor content of the cell volume, such a high percentage of nuclein indicates that also the cytoplasm of the fungi may apparently harbour nucleic acids which could be demonstrated microchemically for the cytoplasma of yeast cells[125].

Considering the cyanobacteria Fischer[126] reports: "I suggest that nucleic substances are also present in cyanobacteria, although not formed into specific structures but lying dispersed within the cytoplasm (that is, in his terms, within the central body). Zacharias[127] likewise confirms the presence of substances among the central part of the cell which does react differently from the nuclein of the cell nucleus.

The fact that the mykoplasma is especially rich in nucleoprotein comes from a comparison of digestible and non-digestible proteins of fungi which entirely consist of mykoplasma[128] and that of plants[129] where the mykoplasma of the nucleus and that of the plastids appears as strongly diluted by the amoeboplasma, that is, by the cytoplasm surrounding the cell nucleus. This can be seen in the following side by side tables. [337]

Fungi	N of the indigestible protein		Plants	N of the indigestible protein	
	in % of dry weight	in % of total N		in % of dry weight	in % of total N
Agaricus, procerus, cap	7.4	20.4	Poppyseed cake	0.706	—
" campestris, cap	16.7	16.0	Sesame cake	0.406	—
" " stem	8.0	18.0	Soybean	0.270	—
Lactarius deliciosus	6.8	33.8	Peanut cake	0.345	—
" torminosus	11.8	40.0	Copra cake	0.254	—
Cantharellus cibarius	4.0	54.6	Rapeseed cake	0.677	—
Boletus edulis, cap	4.3	16.9	Cottonseed cake	0.583	—
" " stem	5.3	20.3	Rice flour	0.409	—
" scaber, cap	6.5	27.2	Rice meal fodder	—	2.106
" " stem	9.6	28.3	Palm cake	—	2.520
" luteus, stem	3.8	42.2	Cottonseed cake	—	7.401
Polyporus ovinus	6.3	46.6	Coconut cake	—	3.549
Hydnum imbricatum	5.0	29.8	Rapeseed cake	—	5.443
" repandum	9.3	44.0	Peanut	—	8.132
Sparassis crispa	6.8	37.4	Lupin	—	7.839
Morchella esculenta	2.5	38.1	Malt sprouts	—	4.167
Lycoperdon bovista	5.2	22.5	Vegetable ivory	—	0.619
Mean:	**7**	**33**	Mean:	**0.456**	**6**

Of course, not the entire mass of indigestible proteins consists of nucleoprotein, in the same way as not every nucleoprotein is indigestible in acidified pepsin. Nevertheless, the aforementioned numbers are of special interest for our

[124] Stutzer, A., Zeitschrift f. physiol. Chemie, Vol. 6, 1882, p. 572.

[125] Janssens, Fr. et Leblanc, A., La cellule, Vol. 14, 1898, p. 203. – Annales de microgr., Vol. 10, 1890, cited from Lafar, Handb. d. techn. Mykol., Vol. I, p. 298.

[126] Fischer, A., Die Zelle der Cyanophyceen. Botan. Ztg., Series I, 1895, p. 118.

[127] Zacharias, E., Über die Zellen der Cyanophyceen. Botan. Ztg., Vol. 48, 1890, p. 66.

[128] Czapek, Fr., Biochemie der Pflanzen, Vol. II, Jena 1905, p. 79.

[129] Czapek, Fr., l. c., p. 154, according to the investigations of Klingenberg and Stutzer.

purpose, whereby the absolute amounts of nucleoproteins are of less significance than the comparison of the two groups of organisms in this regard. From these data we may conclude that organisms consisting of pure mykoplasma (fungi) contain on average 33% insoluble proteins, [337] whereas in those organisms in which the mykoplasma is present only as the cell nucleus, such proteins account for only 6%. This difference must be due at least in part to the unequal amounts of nucleoproteins present in both cases.

From all this we conclude that the mykoid organisms and the nuclei of the amoeboid organisms are rich in nucleoproteins. But does also the amoeboplasma contain it? Let us see what the experts say.

V e r w o r n [130] states "It turns out that the nucleus primarily harbours the phosphorus containing compounds of the proteins and especially nucleins, which within the protoplasma appear to be altogether absent". G u r w i t s c h [131] echoes "that the strict localization of the chromatin to the nucleus has to be maintained", whereby he uses the term chromatin exclusively for such bodies that contain genuine nuclein and which must be strictly separated from the pseudo- or paranuclein as constituents of the cytoplasm. "Only the latter, identical with nucleoalbumines and therefore not representing real nucleic acids or xanthine bases containing bodies, are found inside the cytoplasm, according to numerous investigations" [132].

[338] Therefore, according to numerous chemical investigations, one encounters true nucleins (that is, nucleoproteins), exclusively among the mykoplasma, that is, inside the nuclei, in bacteria, fungi and cyanobacteria[133]. In typical amoeboplasma, that is, in the cytoplasm itself, they do not occur at all. There they are represented by nucleo-albumins.

If one compares the presence of nucleoproteins among the free living as well as the symbiotically living mykoids with their total absence in the amoeboplasma (cytoplasm), we cannot otherwise state that both plasma lineages exhibit a profound and essential difference among one another. Less essential, but also worthwhile to notify, is the circumstance that the mykoplasma alone is capable of synthesizing various enzymes. The capability of bacteria to synthesize enzymes is generally known, but also fungi possess this ability to high extent[134]. If one ascertains the production of enzymes also in animals and plants, as it becomes more and more evident, the cell nucleus, and again the mykoplasma, appears as the primary source of enzyme production. It is almost impossible to put forward a single proven case where the enzyme would have been produced by the cytoplasm itself.

In addition, we can turn our attention to another chemical body typical for the mykoplasma, especially as it is found in mykoids, although one encounters it occasionally in animals, too. This is glycogen.

E r r e r a [135] was the first to state that starch and sugar, acting as reserve substances in plants, is replaced by glycogen in fungi. Glycogen and similar substances have also been found more than once in bacteria, for example in *Granulobacter polymyxa*[136], in *Azotobacter*, and in cyanobacteria, respectively[137].

[339] Further evidence for the different chemical composition of the two plasma lineages is found in the differences of the initial assimilation products among mykoids and amoeboids. In all green plants saccharose is widespread. It represents, as many physiologists like B r o w n and M o r r i s suggest, the initial photosynthetic product following assimilation of CO_2. In all parts of green plants exists an enzyme called i n v e r t i n which converts saccharose into another sugar that is used as material to synthesize starch and inulin by polymerization of sugar molecules. In contrast, the fungi typically possess the sugar trehalose instead of saccharose (which sometimes may also be present)[138], and the enzyme invertin is replaced by a different enzyme – trehalase[139].

In this chapter it has become evident how numerous the gaps in our knowledge are with respect to the chemical composition of cells as well as which experiments are needed and how their results might impact the theory of two plasma lineages. These are now the themes to which I would like to direct the attention of chemists and physiologists:

1. To determine the phosphorus (P_2O_2) content in the ash of a) cyanobacteria, b) bacteria, c) pure amoeboid cytoplasm without nuclei[140], d) purified nuclei without traces of cytoplasm.
2. To determine the phosphorus (P_2O_2) content inside the cell (cytoplasm together with nucleus), but without cell wall in fungi and to compare it with corresponding experiments in plants and animals.
3. To explain the richness in phosphorus in the muscle ash.
4. To elucidate microchemically the composition of plastids a) in relation to phosphorus amount in general and especially b) in relation to nucleic acids and c) in relation to nucleoproteins. In the same way the nucleolus should be investigated.
5. [340] To determine the content of nuclein and especially that of nucleoprotein in a) cyanobacteria, b) fungi, c) bacteria, d) in pure cytoplasm[141].

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[130] V e r w o r n , M a x , Allgemeine Physiologie. Jena 1901, p. 121.

[131] G u r w i t s c h , A., Morphologie und Biologie der Zelle. Jena 1904, p. 163.

[132] G u r w i t s c h , l. c., p. 163.

[133] It would be extremely interesting to carry out dedicated experiments to clarify the question of whether real nucleins are contained in the cytoplasm of fungi. As far as is known here, such an investigation has not yet been performed, with the exception of the aforementioned reference to the presence of nucleic substances in the cytoplasm of yeast fungi (see p. 335).

[134] The fact that plastids produce enzymes can be seen from starch grains that they contain, which often appear as having been gnawed upon and partly "digested" by them.

[135] E r r e r a , L., L'epiplasma des Ascomycètes et le glycogène des végétaux. – Thèse. Bruxelles 1882.

[136] C z a p e k , Fr., Biochemie der Pflanzen, Vol. I, 1904, p. 238.

[137] F i s c h e r , A., Die Zelle der Cyanophyceen. Botan. Ztg. 1905. – It would be very interesting to find out whether glycogen is present in the pyrenoids of some brown algae, especially in those of diatoms, which incidentally, would not be difficult to establish, since we have a very characteristic colour assay for glycogen.

[138] C z a p e k , Fr., Biochemie der Pflanzen, Vol. I, 1900, p. 229 and 501.

[139] B o u r q u e l o t , E., and H é r i s s e y , H., Sur la tréhalose: sa presence générale chez les champignons. Comptes Rend. Acad. Sc. Paris, Vol. CXXXIX, 1904, p. 874.

[140] The separation of the pure cytoplasm from the nucleus for a specific purpose does not have insurmountable difficulties; there are several methods for this.

[141] It may be possible to obtain a considerable amount of pure cytoplasm from the sea urchin's eggs for analysis by excluding the cell

VII. The relationship to toxins and general robustness.

The robustness of the mykoplasma against toxic substances and especially against all forms of harmful external conditions is no less than astounding, and indicates that this plasma must be of totally different structure when compared with the highly sensitive amoeboplasma, which succumbs to even the slightest detrimental conditions.

If we consider aquatic life, starting with absolutely clear water and proceeding through intermediate states ending with the dirtiest and stinking sewers as it has been done in very systematic studies by Kolkwitz and Marsson[142], we see a gradient. In clear water, the amoeboid organisms represent the only organisms or dominate over the mykoids, but decline in numbers the more dirty the water becomes. Concomitantly, the mykoids represented by bacteria and cyanobacteria become more abundant the dirtier the water becomes, until at the very end spoiled and stinking water bodies harbour only bacteria and cyanobacteria.

In order not to remain without evidence I would like to present some data taken from the above mentioned article of Kolkwitz and Marsson. These authors separate the organisms — in the cited article only plants — according to the degree of water fouling that they are able to tolerate. They designate organisms that can only live in absolutely pure, clean water as katarobs (which are not considered here). The oligosaprobes require rather clean water, followed by the mesosaprobes and finally the polysaprobes, which are the least choosy with regard to water purity. I have arranged the percentages of mykoids and amoeboids found within these categories into the following table.

From this table it becomes evident that the number of amoeboid organisms decreases with decreasing water quality, [341] whereas the number of mykoids gradually increases, thereby indicating that mykoids are more robust than amoeboids.

General number of	Oligosaprobes (least fouled water)	Mesosaprobes	Polysaprobes (most fouled water)
Mykoids	21 i.e. 13%	27 i.e. 21%	19 i.e. 90.5%
Amoeboids	137 i.e. 87%	104 i.e. 79%	2 i.e. 9.5%
Total organisms	158	131	21

Mycological specialists are quick to point out the enormous robustness of fungi. "According to Clark fungi are generally more able to withstand unsuitable conditions in comparison with higher organisms"[143]. Similarly, Schmidt and Weis[144] confirm that with regard to the medium in which they grow, bacteria generally "occupy a special position when compared to other plants".

Before we get into details, let us first consider the effects of toxic substances.

It is common knowledge that animals and plants react most sensitively to minimal doses of mercuric chloride. For instance, Miquel[145] who investigated the effect of mineral poisons on diatoms found that the following negligible doses of different toxic substances are lethal.

	Still alive on exposure	Dying on exposure
Mercuric chloride	$^1/_{100\,000}$	$^1/_{40\,000}$
Copper sulfate	$^1/_{75\,000}$	$^1/_{50\,000}$
Zinc sulfate	$^1/_{40\,000}$	$^1/_{30\,000}$
Arsenic acid	—	$^1/_{20\,000}$

According to Davenport and Nealy[146] even a solution of 0.0001% mercuric chloride kills some infusoria (*Stentor*), but a 0.001% solution will kill them quickly. For higher animals (according to Behring) one part of mercuric chloride in relation to 60.000 parts of animal weight is lethal. On the other hand, for bacteria, a relation of one part to 100 parts of serum only leads to developmental arrest. This may indicate that mercuric chloride is six times more toxic for animals than for bacteria[147]. Kossjakow succeeded to get bacteria gradually used to even higher doses of poison as shown in the following table: [342]

nuclei using existing methods (by shaking). Some data already exist concerning the amount of nucleo-proteins in the cell nuclei (Kassel).

[142] Kolkwitz, R. and Marsson, M., Ökologie der pflanzlichen Saprobien. Ber. d. deutsch. botan. Gesellsch., Vol. XXVIa, 1908, p. 505.

[143] Massee, Text-Book of Fungi. London, 1906, p. 127.

[144] Schmidt, Johs and Weis, Fr., Die Bakterien, 1902, p. 104.

[145] Just's Jahresbericht für 1892, p. 175.

[146] Davenport, C. B. and Nealy, H. V., Acclimatisation of Organisms to poisonous Chemical Substances. Arch. F. Entwicklungsmech. d. Organismen. Vol. II, 1896, p. 570. – Also according to Brokorny, Th., Arch. f. Physiol., Vol. CX, 1905, p. 203.

[147] Schmidt, Johs and Weis, Fr., Die Bakterien, 1902, p. 171.

	‰ Borax range	‰ Boric acid range	‰ $HgCl_2$ range
Bacillus subtilis	11—18	9—11	0.07—0.10
Bacterium anthracis	4—7	6—8	0.05—0.07
Bacillus (Thiothryx) tenuis	16—21	9—11	0.10—0.17

Although the amoeboplasma already dies at 0.0001% mercuric chloride, *Bacillus subtilis* withstands 0.01%. This means that the mykoplasma is 100-fold more resistant than the amoeboplasma; *Bacillus tenuis* even tolerates 0.017% of the solution. But this is nothing when compared with the resistance of actinomycetes, a group of organisms positioned between bacteria and fungi. *Actinomyces odorifer* withstands the following unbelievably high toxin concentrations[148]:

NaCl	Carbolic acid	H_2SO_4	$AgNO_3$	$HgCl_2$
Concentrated solution	5%	0.1%	0.1%	0.1%

And while the amoeboplasma dies already at 0.0001% mercuric chloride, *Actinomyces* tolerates up to 0.01% of the poison, indicating that the mykoplasma is 100-fold more resistant than the amoeboplasma. If one believes Johan-Ohlson[149], *Aspergillus niger* even tolerates 1% mercuric chloride solution.

Similar results for the mykoplasma were obtained from another toxic substance called lapis. According to Bokorny[150], infusoria fall victim to 0.001% $AgNO_3$, while *Actinomyces odorifer* resists a 100-fold enriched solution, or 0.1%. This also applies to other toxic substances and harmful conditions which every amoeboplasma would not have survived long since.

Alcohol, for instance, kills every animal and plant immediately. However, Russ[151] has shown that desiccated bacteria do not suffer from alcohol, even from absolute alcohol, while bacterial spores are entirely resistant against alcohol of any concentration. "Absolute alcohol has almost no disinfecting influence on bacterial spores"[152].

[343] The same results were noticed for fungi. Hoffmann[153] reports that Schmitz observed spores of *Peziza repanda* germinating after being stored in absolute alcohol for 24 hours.

Bacteria are completely insensitive to solutions of sodium chloride. It is beyond doubt that no animal or plant is able to live for a longer period in 25% salt solution, even less in concentrated salt[154]. — By contrast, many bacteria live and propagate normally in 10% salt solution, in which they continue to secrete their typical enzymes[155]. Fischer emphasizes that such bacteria are fully permeable in that they allow the salt to completely pass through their plasma membrane. *Penicillium* not only survives in 13% salt solution, it is even able to grow[156].

But that is not all. Lewandowsky[157] cultivated bacteria in 25% salt solution where they lived rather well. And quite a number of bacteria can survive in even higher concentrated solutions for many weeks, as for instance *Bacillus coli communis* for 6 weeks[158], without losing their viability.

Bacterial spores are even more resistant: those of *Bacillus anthracis* are able to survive in concentrated NaCl solutions for months, those of the diphtheria agent for three weeks[159].

[344] Bacteria are even able to live in herring brine, though they do not multiply[160].

Apparently the mykoplasma of bacteria must be of a different structure compared to that of the amoeboplasma of animals and plants, considering that it is able to live in media like herring brine or even concentrated salt solutions.

One of the strongest poisons for the amoeboplasma is $CuSO_4$. Diatoms, for instance, as we have seen in the beginning of this chapter, already die at 1/50.000 of this salt, whereas according to Nägeli[161] *Spirogyra* and some other algae are even more sensitive to this poison and do suffer in solutions

[148] Rullmann, W., Die Eisenbakterien. Der Kreislauf des Schwefels, in Lafar, F., Handb. d. techn. Mykologie. Vol. III, Jena 1904, p. 212.

[149] Just's Jahresbericht, 1886, p. 475.

[150] Bokorny, Th., Nochmals über die Wirkung stark verdünnter Lösungen auf lebende Zellen. Pflüg. Arch. f. Physiol. des Menschen. Vol. CX, 1905, p. 203.

[151] Russ, v., Zur Frage der Bakteroidie durch Alkohol. Centralbl. f. Bakter. (Series I), Vol. XXXVII, 1904, p. 115.

[152] Minervini from Schmidt, Johs and Weis, Fr., Die Bakterien. Jena 1902, p. 173.

[153] Hoffmann, H., Untersuchungen über die Keimung der Pilzsporen. Pringsh. Jahrb. f. wiss. Botan., Vol. II, 1860, p. 331.

[154] In Oltmanns (Morph. und Biologie der Algen, Vol. II, p. 187) we find the following information regarding the resistance of the algae: "In cultures, green algae, which are relatively robust (that is, relative to red algae), were often observed in concentrated salt solutions. Stange grew *Chlamydomonas marina* in a 23% salt solution und *Pleurococcus* spec. in 12% nitrate solution. Wiplel reported similar for *Pleurococcus*, whereas Spirogyras and Vaucherias were less robust. A. Richter succeeded in growing different freshwater green algae in fairly concentrated salt solutions" …

But here Oltmanns adds: "From the experiments of Richter and Drewsit is apparent, that the algae do not permanently tolerate high salt conditions."

It would be difficult to find an alga that can live in herring brine or conentrated salt solutions, even for a short time.

[155] Fischer, A., Botan. Ztg. 1905, p. 104.

[156] Eschenhagen, Einfluss der Lösungen verschiedener Konzentrationen auf Schimmelpilze. Dissert. Leipzig 1888.

[157] Lewandowsky, F., Arch. f. Hyg., Vol. XLIX, 1904, p. 47.

[158] Fischer, A., Vorlesungen über Bakterien, 1903, p. 29.

[159] Freitag, C., Zeitschr. f. Hygiene, Vol. XI, p. 60, from Czapek, Biochemie der Pflanzen, Vol. II, p. 900.

[160] Fischer, A., Vorlesungen der Bakterien, 1903, p. 29.

[161] Oltmanns, Fr., Morphologie und Biologie der Algen, Vol. II, Jena 1905, p. 184. – See also: Nägeli, Olygodynam. Erscheinungen in lebenden Zellen. 1893.

containing one part $CuSO_4$ in 50 million parts of water, according to Bokorny[162] even in dilutions of one to one hundred million.

Now we will see how fungi respond to this poison.

Bokorny[163] states "Some fungi are relatively insensitive against $CuSO_4$, contrary to algae and infusoria which become easily damaged". And De Bary[164] states "I have investigated thalli of *Penicillium glaucum* a foot in length that have formed on the surface of $CuSO_4$ solutions used for galvanoplastic purposes", similar to Berkely[165] who found this fungus upon solutions of ferric sulfate. Hoffmann[166] observed opulent thalli of *Penicillium glaucum* in rich spore formation upon the surface of saturated arsenic acid. This was also found by Jaeger[167]. Pulst[168], who carried out many experiments investigating the resistance of moulds against $CuSO_4$, reported that *Penicillium glaucum* is remarkably resistant in this respect. He also recalls the "rather low resistance of *Mucor* in general" [345] and of the impeding influence of this poison on the development of *Mucor* which is the most sensitive of the three fungi (*Aspergillus*, *Botrytis* and *Mucor*). But *Mucor* is a phycomycete, *[Mucor is a fungus]* a plastid deficient alga (amoeboid) in contrast to more resistant fungi that are true mykoids. The same behaviour was also observed regarding the influence of H_2S and CO_2. — Bacteria (for example *Beggiatoa*) and cyanobacteria incorporate H_2S, which for animals and plants is highly toxic. *Mucor* (amoeboid) already suffers at 33% CO_2, for the fungus *Penicillium* only levels above 80% cause toxic effects. Many bacteria, however, live in pure CO_2 as well as they do in air[169].

Numerous experiments on the effect of various poisons carried out by Bokorny[170] confirm the remarkable resistance of the mykoplasma as seen from the table below. *[The table on p. 345-346, printed here on the following page, appears here]*.

Particularly remarkable is the difference between the mykoid and amoeboid plasma regarding toxins like hydrogen cyanide, morphine, strychnine which are especially poisonous to the latter. Schmidt and Weis[171] write: "The effect of various poisons remains most mysterious, ...while they are lethal for a given organism even in smallest doses, they may be harmless for others even at high doses. Thus hydrogen cyanide and the alkaloids strychnine, morphine and others which belong to the most dangerous poisons for higher animals may serve as growth substrate for yeasts and bacteria".

Pfeffer[172] reports on the remarkable fact that some fungi take up amygdaline or even potassium cyanide as growth substrate and use these substances, which are extremely toxic for animals, as a source to obtain their required nitrogen[173].

By contrast, according to Klebs[174] these alkaloids, especially strychnine, are harmful for unicellular algae like *Euglena* and *Phacus* and also for higher plants even at concentrations of 0.05%.

[347] Also unusual is the tolerance of bacteria to gastric acid as pointed out by Ruzicka[175]. "The anthrax bacterium that was subject to gastric juice for 51 days and more offers almost the same image to the eye in the microscope as bacteria freshly taken from the living culture." In this respect bacteria behave identically to cell nuclei which, as everybody knows, are almost impervious to gastric acid. About which cell, whether it comes from an animal or a plant, can one make the same statement? [346]

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[162] Bokorny, Th., Nochmals über die Wirkung stark verdünnter Lösungen auf lebende Zellen. Pflüg. Arch. f. Physiol. des Menschen. Vol. CX, 1905, p. 204.

[163] Bokorny, l. c., p. 203.

[164] De Bary, Beiträge zu Morphologie und Physiologie der Pilze, Flechten und Myxomyceten. 1866, p. 214.

[165] Berkley, Outlines, p. 30 (after De Bary, Beiträge zu Morphologie und Physiologie der Pilze, Flechten und Myxomyceten. 1866, p. 214.)

[166] Hoffmann, H., Untersuchungen über die Keimung der Pilzsporen. Pringsh. Jahrb. f. wiss. Botan., Vol. II, 1860, p. 330.

[167] Jaeger, Wirkung des Arseniks auf Pflanzen. Stuttgart 1864 (after De Bary, Beiträge zur Morphologie und Physiologie der Pilze, Flechten und Myxomyceten. 1866, p. 214.)

[168] Pulst, C., Die Widerstandsfähigkeit einiger Schimmelpilze gegen Metallgifte. Pringsh. Jahrb. f. wiss. Botan., Vol. XXXVII, 1902, p. 214 and 215.

[169] Chopin, Flora, 1902, Supplementary Vol. p. 348.

[170] Bokorny, Th., Nochmals über die Wirkung stark verdünnter Lösungen auf lebende Zellen. Pflüg. Arch. f. Physiologie des Menschen. Vol. CX, 1905, p. 174. – In Borkony's work, one will come across some observations that contradict the general view about the effect of poisons on amoeboid and mykoid organisms. From this work it becomes evident how important it is to formulate a scientific problem. — If the purpose of this work had been, for example, to test my theory of two plasma lineages, it could certainly provide extremely valuable facts for the critical illumination of these lineages. As it stands, however, little can be inferred from it to address the question of interest to us, because in the reported observations of the effect of this or that substance on animals and plants, nowhere were parallel experiments made of the effect on mykoids. Apart from these gaps, the facts cited by Bokorny are often insufficient for our purposes because they do not give any indication of the conditions under which a given toxin acts on an organism. However, knowing these conditions is extremely important, because mercuric chloride loses much of its toxicity in the presence of the smallest amount of proteins, for example. In Bokornys work there are cases where bacteria (always putrefactive bacteria) are more sensitive to toxins than infusoria and algae, but it is possible that the bacteria lived in a medium that was less rich in organic matter than that of the infusoria. – On the other hand, it is possible that if Bokorny did not try to test the effect of this poison only on putrefactive bacteria, which by chance may have proved to be particularly sensitive to the present poison (it is well known that different genera and even different species also have different sensitivities), it is possible that a different relationship of the organisms to some poisons would have been obtained. — It is possible to coincidentally encounter bacteria that perish at temperatures that are still withstood by infusoria, but this does not mean that infusoria are more resistant to high temperatures than bacteria.

[171] Schmidt, Johs and Weis, Fr., Die Bakterien. Jena 1902, p. 171–172.

[172] Pfeffer, W., Pflanzenphysiologie Vol. I, Leipzig 1879, p. 398.

[173] It would be very interesting to carry out extensive and systematic experiments on this subject, including cyanobacteria, whose relationship to toxins has not yet been investigated to any great extent.

[174] Klebs, G., Organisation einiger Flagellatengruppen. 1883, p. 59.

[175] Růzička, V., Weitere Untersuchungen über den Bau und der allgemeinen biologischen Natur der Bakterien. Arch. f. Hygiene, Vol. LI, 1904, p. 307.

	Mykoplasma	Amoeboplasma
Hydrochloric acid.	1% — Applied for 48 hours, does not kill *Bacillus anthracis* (Dyrmont).	0.01% kills *Paramaecium* (Infusor) and zoospores.
Potassium hydroxide.	0.1% does not harm the typhoidal bacterium and 0.14% does not harm the cholera bacteria that live in gelatin (Kitasato)[176].	0.1% currently kills all animals and plants.
Copper sulfate.	0.1% disturbs the growth and assimilation of a yeast species; At 1% mould is growing (Bokorny, p. 204), at 0.05% bacteria are growing.	0.01% kills infusoria. 1:50 000 kills all animals in 2 days (infusoria, rotifers, worms, insect larvae) and all plants (*Cladophora*, *Conferva*, *Spirogyra*, *Vaucheria*). 1:100 000 000 slowly kills *Spirogyra* (l. c. S. 205).
Zinc sulfate.	"The life of rot fungi strangely isn't even hindered completely at 0.1% zinc sulphate" (Bokorny, l. c. S. 209).	0.01% kills infusoria in 24 hours (l. c. S. 209) and even 0.001% slowly kills them. Roots of phanerogams die at 0.02%.

[end Part III, vol. 30, No. 10, May 15, p. 347; begin Part IV, vol. 30, No. 11, June 1, p. 353]

(Conclusion).

To explain the remarkable ability of bacteria and fungi, to withstand the harmful effects of poisons like $CuSO_4$, $FeSO_4$, KCN etc., it has been proposed that these toxic substances do not traverse into the cytoplasm in that they are held back by the outer cell wall or the outermost plasmatic layer. Such an explanation is, however, incorrect in certain cases, as for instance highly concentrated salt solutions penetrate the bacterial cell wall. In a similar way, if substances like KCN, morphine, strychnine, serve as food for mykoids, they must find their way into the interior of the cell. This explanation is particularly unsuitable with respect to bacteria "which are able to take up dissolved substances by diffusion more easily and rapidly than other cells"[177]. [354] In fact, it is entirely inadmissible to explain the resistance against toxins in such organisms, which "take up dissolved substances by diffusion more easily and rapidly than other cells" by suggesting that they do not allow toxins to penetrate the cell wall!

However even if it were to be proven that the above mentioned toxins cannot penetrate the cell wall, that would still not diminish the importance of the aforementioned observations, since then there must exist two sharply distinguishable types of plasma, one of which is able to build up a cell membrane or outer protoplasmic layer that easily lets 1/50,000,000 $CuSO_4$ through and in the other that creates such membranes as do not allow toxins to penetrate even at such high concentrations used in the galvanoplastic.

The considerable resistance of the mykoplasma versus the amoeboplasma can also be seen with respect to the mode of nutrition and the selection of suitable food. The amoeboplasma calls for very delicate food, its menue consisting of protein, protoplasma, fat, starch and other carbohydrates. The mykoplasma eats everything possible and impossible and is even satisfied by rough and undigestible food, a diet that would definitely kill every kind of amoeboplasma. — Benecke[178], for example, found a bacterium (*Bacillus chitinovorus*) feeding on chitin. The well known french bacteriologist Miquel[179] observed bacteria that feed on rubber while assimilating a part of it and excreting H_2S. Rahn[180] showed that a fungus (*Penicillium*) can live from paraffin or paraffin-like carbohydrates, using these substances as a carbon source. There are also fungi belonging to the Ascomycetes that use horn (antlers) as food source; *Onygena equina* and *Onygena corvina*[181] are members of this group. We have also seen that the mykoplasma feeds on HCN, KCN, morphine, strychnine, and from chapter IV we have seen that the mykoplasma, and only the mykoplasma, is able to live on inorganic salts and gases, from which they produce proteins.

[355] Such profound nutritional differences can only be manifest in two plasmas that are fundamentally different from one another in their innermost nature.

We became acquainted with the extraordinary resistance of the mykoplasma against high temperatures and have noticed its ability to live without oxygen in chapters II and III. We now come to the conclusion that the mykoplasma is distinguished from the amoeboplasma by its resistance and robustness in general and by its greater ability to withstand harmful physical and chemical factors.

[176] However, it should be noted that it was not possible to detect the presence of cellulose in some phycomycetes. In other cases the question remains controversial. Mangin, for example, found cellulose in *Mucor*, but van Wisselingh did not.

[177] Fischer, H., Die chemischen Bestandteile der Schizomyceten und der Eumyceten, in Lafar, Handbuch der technischen Mykologie, Vol. I, Jena 1904, p. 224.

[178] Benecke, W., Über *Bacillus chitinovorus*, einen Chitin zersetzenden Spaltpilz. Botan. Ztg. 1905, Series I. p. 227.

[179] Perrier, Les colonies animales. 2. Edition, 1898, p. 39.

[180] Rahn, O., Centralblatt für Bakteriologie (II), Vol. XVI, 1906, p. 382.

[181] Ward, H., Marshall, *Onygena equina* Willd, a horn destorying fungus. Philosoph. Transact. of the Royal Soc. London. Series B, Vol. 191, 1899, p. 269.

VIII. The other differences.

1. The mykoplasma is distinguished from the amoeboplasma by the presence of iron in a chemically fixed state. Reasons to postulate this comes from M a c a l l u m 's[182] investigations, which show that the majority of the bound iron, sometimes its entire mass, is contained inside the nuclei of higher animals and plants, especially within the chromatin net of the chromosomes.

On the other hand, iron is also present in plastids and free living mykoids. It was found in bacteria[183], and according to experiments of R a u l i n [184] and M o l i s c h [185], it appears as an essential constituent, also in fungi. It is generally known that without iron typical plastids cannot develop: lacking iron the plant becomes chlorotic, develops weakly and eventually withers. The chemical analysis of bacteria and fungi exhibits iron as well[186]: vinegar bacteria contain 8.15% Fe_2O_3, lichens 5.5–6.6%, mould spores 5%. In the majority of cases, however, the iron content is less prominent as demonstrated above and usually accounts for less than 1%, though in truffle it increases up to 5% and this amount remains constant even in iron-poor soil.

[356] Should the observations of J u s t u s [187] be correct, that each nucleus contains iodine, it appears possible that the presence of this element may also reflect a specific character of the mykoplasma.

2. The mykoplasma of the free living mykoids is always surrounded by a cell wall, the amoeboplasma is often naked. But even in those cases where the amoeboplasma is surrounded by a cell wall as in plants, one encounters the deep differences between the cell walls of mykoids and amoeboids. Plants contain a cell wall made up of carbohydrates, mainly cellulose. This peculiarity led B o n n i e r and L e c l e r c d u S a b l o n [188] to point out that the ability of plants to produce cellulose is one of the major differences among animals and plants. „La présence ou l'absence de la cellulose est encore le moins mauvais des critériums que nous ayons examinés."

The mykoids possess a completely different cell wall. It consists of nitrogen-containing substances, in some cases being similar to chitin (chitosan), in other cases coming close to proteins.

The bacterial cell wall is of proteinaceous substances according to S c h m i d t and W e i s [189], similar to the protoplasma, although most authors suggest a rather similar composition as in fungi; earlier reports indicative of the presence of cellulose inside the bacterial cell wall have not been confirmed.

V a n W i s s e l i n g h [190] reports that the fungal cell wall consists of nitrogen and contains substances (chitin according to him), which are lacking in the Saprolegnieae and Peronosporeae, [357] i.e. in the phycomycetes[191], where the cell wall consists of cellulose, also confirmed microchemically by M a n g i n [192].

Finally, considering cyanobacteria, in which we may expect a cellulosic cell wall due to the presence of chlorophyll, K o h l [193] comes to the conclusion that in the majority of cases the cell wall consists of chitin with the exception of heterocysts where it is made out of cellulose.

3. In addition to all the chemical and physiological differences that we have listed distinguishing the mykoplasma and the amoeboplasma, one may still direct the attention towards certain morphological characters. Whoever compares the peculiar fruit bodies of cap mushrooms, gastromycetes, or of the white rot fungi with a true plant, be it an alga, a moss, fern or an angiosperm, must immediately recognize the enormous differences between the two with respect to their morphology.

The world of fungi with its bizarre shapes gives the impression of a peculiar and foreign appearance, as if these organisms are not from our planet but from some other world. No other plant organism gives such an impression.

But also the inner morphology, i.e. the anatomy of both kingdoms, that of the plant and that of the fungal kingdom, opens up a profound and principle difference[194].

Plants are made of true tissue, fungi never contain tissues. Starting with the simplest fungi and ending with the most elaborated ones all fungi are made up of interwoven hyphae or filaments which all grow simultaneously, [358] explaining the unusually rapid growth typical for fungi.

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[182] M a c a l l u m , A., On the distribution of assimilated iron compounds other than Haemoglobin and Haematin, in animal and vegetable cells. Quart. Journ. of microsc. Sc. Vol. 38, 1896.

[183] S t o c k l a s a for *Bacillus megaterium*, see L a f a r , Handb. d. techn. Mykologie, Vol. I, 1904, p. 397.

[184] R a u l e i n , Anales des Sc. Natur. Sér. V, Vol. XI, 1869, p. 93.

[185] M o l i s c h , H., Die Pflanze in ihren Beziehungen zu Eisen. Jena 1892. — However, W e h m e r believes that iron is not absolutely necessary for the development of the fungi (W e h m e r , C., Zur Frage nach dem Werte der einzelnen Mineralsalze für Pilze. Ber. d. deutsch. Botan. Gesellsch., Vol. XIII, 1895, p. 257).

[186] F i s c h e r , H., Die chemischen Bestandteile der Schizomyceten und der Eumyceten, L a f a r , Handbuch der technischen Mykologie, Vol. I, Jena 1904, p. 227.

[187] J u s t u s , J., V i r c h o w ' s Archiv, Vol. CLXX, 1902, p. 501, ibid. Vol. CLXXVII, 1907.

[188] B o n n i e r , G. and L e c l e r c d u S a b l o n , Cours de Botanique. Vol. I, Paris 1905, p. 16.

[189] S c h m i d t , Johs und W e i s , Fr., Die Bakterien. Jena 1902, p. 21 and 22.

[190] W i s s e l i n g h , C. v a n , Mikrochemische Untersuchungen über die Zellwände der Fungi. Jahrb f. wiss. Botanik, Vol. XXXI, 1898, p. 619. — See also the numerous studies on this subject by W i n t e r s t e i n , Ber. d. deutsch. bot. Ges. 1893, 1894, 1895, also in Zeitschr. f. physiol. Chemie, Vol. XIX, 1894 and 1895 and the work by I w a n o f f .

[191] Incidentally, one sometimes encounters chitinoid substances also in the mucoid-like phycomycetes. See B a c h m a n n in Pringsh. Jahrb. f. wiss. Botanik, Vol. XXXIV, 1900.

[192] M a n g i n , L., Comptes Rend. d. l'Acad. d. Sc. Paris, Vol. XVII, 1893, p. 816. – Incidentally, the microchemical methods are not very reliable, which is why M a n g i n sometimes comes to wrong conclusions, for example regarding the presence of cellulose in the lichens *Usnea barbata*, which v a n W i s s e l i n g h can not confirm. In the case of lichens, the last author was able to establish without any doubt the presence of chitin in the spore walls.

[193] K o h l , F., Organisation und Physiologie der Cyanophyceenzellen. 1903.

[194] And it is not surprising that this is so, because, as C l a u d e B e r n a r d has long claimed, the morphological difference is nothing other than the consequence and manifestation of chemical differences. In the botanical field, this idea was developed by S a c h s (Stoff und Form der Pflanzenorgane. Arb. d. botan. Inst. in Würzburg. Issue 3, 1880, p. 452 ff.). — But see the criticism of this theory by V ö c h t i n g (Bot. Zeit. 1880, p. 609 ff. and P r i n g s h . Jahrb. 1885, p. 24 ff.) and von R e i n k e (P r i n g s h . Jahrb. Vol. XXXI, 1898, p. 252 ff.)

4. We have good reasons to assume that the mykoplasma reveals a much more complex structure than the amoeboplasma.

The reason is founded in the role that the mykoplasma plays with respect to inheritance. In case my theory regarding the origin of the cell nuclei is correct, which I would like to propose in the forthcoming article, the mykoplasma appears as the carrier of inheritance: This is because the chromosomes and namely the chromatids can only be made of this kind of plasma, but not of the amoeboplasma. Let us now remember which complex characters are being inherited by the chromatids, especially in higher organisms. Not only all details of their organization, not only smallest spots of coloration, but also psychic nature, disposition, talents are being inherited from one generation to the other and therefore must reside within the chromatids. If we take into account all this we have to allow for such complexity in the construction of chromatids which nearly comes close to impossibility[195)].

And similarly, we have no reason to entertain the notion of a similar complexity for the amoeboplasma.

IX. Conclusions from the theory of two plasma lineages.

In the previous chapters we have seen that there are a number of profound differences between the two groups of organisms which we named mykoids and amoeboids. We have also seen that each group is referred to one type of plasma revealing such divergent characters that we have to accept fundamental differences in the structure of these two kinds of cytoplasm.

[359] From this we are forced to accept an unambiguous duality of the living world instead of being homogeneous.

From that, however, follow numerous logical consequences, which we now consider briefly.

If there are two fundamentally different types of plasma regarding their properties and, as a consequence, two worlds of living organisms, this can only be explained by the fact that both plasma lineages originated independently of each other under different conditions at different eras during Earth's history.

The history of Earth may be divided into four epochs as far as they are related to the origin of life and of organisms. These geological eras probably comprise very different periods of time.

E p o c h I : Fiery glowing state of the Earth's surface.

E p o c h I I : The Earth is no longer glowing, but still very hot (more than 100 °C) and therefore absolutely dry.

E p o c h I I I : The surface of the Earth is covered with boiling or hot water with temperatures of 50–100 °C.

E p o c h I V : The water temperature falls below 50 °C.

In which of these periods could life have emerged?

According to P f l ü g e r [196)] its initial stages could have been related to cyan molecules and some other radicals of proteins at times when the Earth still remained in its fiery-glowing state, because such substances require very high temperatures to be formed. But life itself, that is, living protoplasma, could originate only after water appeared on Earth's surface. This we may conclude from the following:

1. We do not know of any absolutely dry organism; all living beings require a certain amount of humidity, though not externally but internally.

2. All chemical processes operate better in water or solutions and thus it is quite natural to assume that such a complicated chemical process like the formation of the living protoplasma occurred in water under conditions which are much more suitable than within a dry medium. Thus, organisms were only able to appear within the third or fourth period of the Earth's history. But in which of them?

The properties of the mykoplasma described above allow us to answer this question in more detail than was previously possible. [360] The mykoplasma could have easily originated within the third period, when the water was still hot, saturated with minerals and devoid of oxygen. The rough conditions under which this plasma originated would explain its remarkable properties, its unusual tolerance of high temperatures, its tolerance of concentrated solutions of various harmful substances, its ability to live without oxygen and to synthesize its own proteins exclusively from minerals and so on.

What was the nature of the first organisms that appeared on Earth during this epoch? Doubtlessly, they were among the most primitive ones that we know today — the bacteria. This becomes evident from the following table, in which the requirements for life among organisms that originated within the third period are contrasted with the morphological and physiological attributes of bacteria which, as it becomes obvious, entirely coincide with those requirements.

[361] This remarkable coincidence of bacterial properties with the demands imposed upon the first very organisms allows us to propose that they were indeed bacteria. Furthermore, since our demands require that the first organisms appeared when the water temperature was higher than 50 °C, our premise that bacteria evolved during the third period of the Earth's history appears well founded. The first living plasma to occur on Earth must have been very robust and fully equipped to withstand the rough conditions on the early Earth. And this plasma was the mykoplasma.

Thus there was a time when bacteria were the only organisms on Earth. The hot, even boiling waters of the ocean, alkaline, enriched with salts, sulfur containing substances, but lacking oxygen, were full of bacteria, which either lived on the sea floor as gelatinous layers, as floating slimy lumps and mats or simply existing suspended as individual cells that clouded the water. — Such conditions persisted on Earth for thousands and hundreds of thousands of years, giving the bacteria time to evolve. From these simply organized biococci various other forms escaped including bigger ones as well as assembled structures. Finally, bacteria gave rise to other, much more highly organized groups of organisms — fungi and cyanobacteria. [360]

[195] It is possible that the extraordinary complexity of the structure of mykoplasma is directly related to another property of this plasma – its immobility. A very mobile substance can never reach the high degree of complexity that a less mobile substance can. And this in turn may be related to the greater density of mykoplasma, which we can tentatively attribute to this plasma and which would explain its great resistance to high temperatures.

[196] P f l ü g e r , Über die physiologische Verbrennung in den lebendigen Organismen. – Pflüg. Arch. f. Physiol., Vol. X, 1875.

Requirements, which necessarily have to be met by the first organisms.	Attributes of the bacteria that match the requirements.
1. Minimal size, inaccessible to the microscope.	1. The bacterial fogs consist of bacteria like organisms that are invisible under the microscope — the biococci[197].
2. Absence of organization.	2. At such a small size, biococci can not have organization, following the law of dependence of organization upon size.
3. Ability to withstand high temperatures close to the boiling point.	3. Bacteria tolerate temperatures up to 98 ° in the vegetative state and up to 150 ° in the reproductive state.
4. Ability to live without oxygen.	4. The vast majority of bacteria can live without oxygen.
5. Ability to synthesize proteins and carbohydrates (the latter without the help of chlorophyll) from inorganic substances.	5. The bacteria are able to synthesize proteins and carbohydrates (the latter without the help of chlorophyll) from inorganic substances.
6. Resistance concerning alkaline solutions, strong saline solutions, sulphur compounds and various toxins.	6. Bacteria tolerate alkaline solutions, highly concentrated saline solutions, hydrogen sulphide, large doses of various toxins.

The theory of the origin of organisms presented here benefits from being fully consistent with Pflüger's hypothesis for the origin of life on Earth, of which Verworn says that there is not a single fact contradictory to it.

Placing the origin of the mykoplasma within epoch III of the Earth's history, which follows as a consequence of the theory of the two plasma lineages, fits in well with Pflüger's theory, to a certain extent being its continuation. If, as usually assumed, life would have originated within the epoch IV, that is, in the period of cooling oceans, [362] an enormous gap would separate the formation of the building blocks required for the formation of living protoplasm from cyanidic and other radicals, whose synthesis requires high temperatures, and the assembly of these radicals into living plasma. My theory avoids such a gap, it allows the continuity of processes that culminate in the synthesis of life *[Lebensbildung]*. At a time when the poles of the Earth had cooled sufficiently that on their surfaces the first boiling water could condense, at the equator the temperature could have been so high as to allow radicals to form and to persist, radicals that, coming into contact with boiling water, formed the first granules of living matter. — This transitional moment, during which remnants of epoch II prevailed while at the poles conditions of epoch III had set in, was probably the moment at which the mykoplasma formed. Before that time, the water required for the existence of life would not have existed. Subsequent to that time, the elements required for the synthesis of plasma, that is the building blocks *[Bausteine]* from which it was formed, could not remain stable, they began to decompose and could not be assembled anew. Because of this, the conditions required for the formation of the living mykoplasma dissipated and the further evolution of life was only possible following the principle: omne vivum e vivo. In this way the most prominent distinguishing character of life arose, namely the ability to propagate, that is, to allow new organisms to emerge using parts of the preceding generation. Without this ability of the first protein particles to grow there would be no life on Earth. — The occurrence of all living mykoplasma thus emerges from growth of the original mykoplasma, as its direct continuation *[als dessen unmittelbare Fortsetzung]*.

Only after the water temperature had dropped below 50 °C, and there was plenty of organic food on Earth in the form of bacteria, could the second type of plasma — the amoeboplasma — emerge. Very different conditions existed during the epoch of its origin. Those conditions were much less inhospitable compared to those at the formation of the mykoplasma. They elicited the very different properties that characterize the amoeboplasma.

[363] This type of plasma probably arose in the form of small clumps[198], as small anuclear Monera that crawled like amoebae on the ocean floor and consumed bacteria, which were present in abundance.

In the majority of cases the bacteria were digested by the Monera, but there must have been such species as well that were able to resist the digestive power of the Monera. Such bacteria remained alive inside the bodies of the Monera where they formed with it a symbiosis; these symbiotically living mircococci, living unordered at first and dispersed within the Moneran cell body, then in the form of a distinct group assembled in the cell's centre and finally surrounding themselves by a membrane *[Häutchen]*, thereby formed the cell nucleus[199]. The cell nucleus opened up completely new possibilities with regard to the further evolution of the Monera. Without this symbiosis the anuclear Monera would have been condemned for ever to remain the same lowly life form that they originally were. Without the penetration of bacteria — these enzyme synthesizers par excellence — into the interior of the originally anucleate Monera, we would have neither animals nor a plant kingdom with the endless diversity of form. That diversity stems from nothing other than the diversity of enzymes that, as we know, stem from the nuclei. Without that symbiosis, the entire organic world would be represented by the

[197] See above: Löffler und Frosch, Berichte der Kommission zur Erforschung der Maul- und Klauenseuche bei dem Institut für Infektionskrankheiten in Berlin. Centralbl. f. Bakter., Series I, Vol. XXIII, p. 371. – Nocard et Roux, Annales del Institut Pasteur, 1898, No. 4. – Errera, L., Recuel de l'Institut botanique, Université de Bruxelles, 1903. – Lafar, Handb. d. techn. Mykol., Vol. I, 1904, p. 32 and 35.

[198] The theory recently presented in this journal, according to which first a continuous mass of organic living matter was created, which then broke into several individual particles, does not withstand critique; it is in direct opposition to the general law of evolution of organisms, according to which evolution increases from small and simple to large and composite (see my course on general botany, in Russian).

[199] The aspect of my theory about the origin of organisms that deals with the cell, its nature and formation, is the topic of a different paper in which facts will be presented that serve as the basis for the sentences that are only briefly expressed here.

vast and extraordinary kingdom of fungi and on the other hand by primitive Monera.

When the cyanobacteria evolved from bacteria — among which already various pigmented species existed: red, yellow, green — by means of increased pigment synthesis, they invaded as new endosymbionts numerous amoebae and flagellates which already existed at the time, descended from the first symbiosis between bacteria and anuclear Monera. This new symbiosis initiated at once the origin of several (from six to nine) independently sprouting, main branches in the tree of the plant kingdom *[unabhängig voneinander sprossende Baumstämme des Pflanzenreiches]*. Such a highly polyphyletic origin of the plant world, [364] which now can be taken as conclusively shown, appears as a consequence of the observation that various cyanobacteria (green, brown, red) invaded various flagellates, some possessing one flagellum, some possessing two identical flagella, and some possessing two non-indentical flagella of differing morphology.

The remaining amoebae and flagellates that did not enter into endosymbiosis with cyanobacteria went on to evolve into animals, thus creating the animal kingdom.

As an additional consequence of the theory of the two plasma lineages we are faced with a new classification of organisms and completely different phylogenetic relationships among individual groups relative to what is generally accepted today.

The first branch to diverge in the organic world as a new kingdom was the mykoid kingdom, consisting of pure mykoplasma. It is the only kingdom that does not appear as the result of a symbiotic event, but evolved on its own from the most ancestral organisms, the urbacteria. The other two kingdoms, the plant and the animal kingdom, emerge as the result of symbiosis; animals resulting from a single symbiosis, plants however — as the result of two symbioses[200]. The new classification of organisms can be expressed as follows:

I. The mykoid kingdom (no symbiosis)	Free living	1. Bacteria 2. Fungi 3. Cyanobacteria
	Symbionts	1. Plastids 2. Chromatin granules of nuclei
II. The plant kingdom (twofold symbiosis)	1. Algophyta	a) Algae (autotrophic organisms) b) Leucophyceae (heterotrophic organisms, Phycomycetes)
	2. Bryophyta	
	3. Pteridophyta	
	4. Spermatophyta	
III. The animal kingdom (single symbiosis).		

As an additional consequence of the new theory of the two plasma lineages follows the need to revise the relationships between some groups of organisms compared to those generally accepted today.

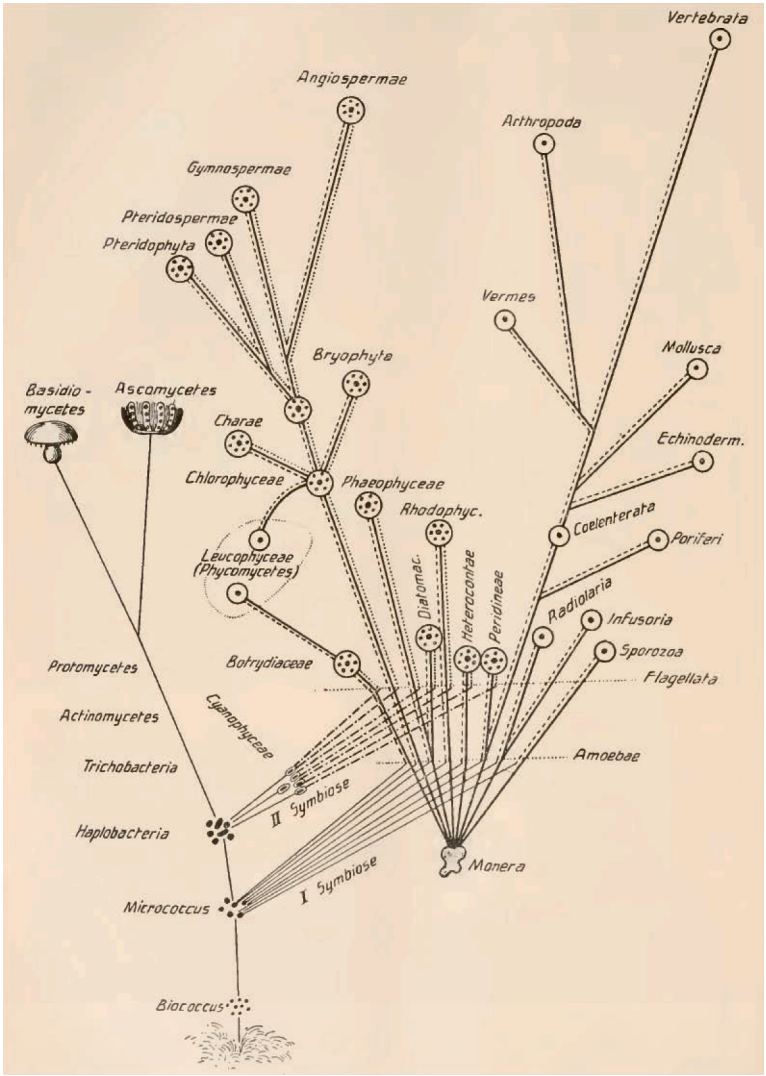
It appears unavoidable to exclude from the fungi the phycomycetes, which De Bary already interpreted as algae that had lost their pigments. [365] Yet De Bary places them among the fungi. How far the phycomycetes are apart from the fungi and how close they are to plants becomes evident from the following table:

Plants.	Phycomycetes.	Mykoids.
1. The plasma is capable of amoeboid movement.	1. The plasma is capable of amoeboid movement.	1. The plasma is incapable of amoeboid movement.
2. Contractile vacuoles present.	2. Contractile vacuoles present.	2. No contractile vacuoles present.
3. Increase their number via zoospores.	3. Increase their number via zoospores.	3. Don't increase their number via zoospores.
4. The cell walls consist of cellulose.	4. The cell walls consist of cellulose[201].	4. The cell walls consist of fungin or chitin.
5. The spores are naked, formed by the fission of protoplasm, occasionally with periplasm.	5. The spores are naked, formed by the fission of protoplasm, no epiplasm.	5. Spores always have a membrane, they are formed by internal deposition of individual parts from the general mass of plasma, epiplasm always present.

[366]

[200] The lichens represent a threefold symbiosis.

[201] However, it should be noted that it was not possible to detect the presence of cellulose in some phycomycetes. In other cases the question remains controversial. Mangin, for example, found cellulose in *Mucor*, but van Wisselingh did not.



Also, morphologically the phycomycetes are close to various types of algae that there can be no doubt that these organisms are not fungi but colourless algae which have lost their plastids due to a saprophytic or parasitic life cycle[202]. Therefore I recognize the phycomycetes as a side branch (more exactly as several side branches) of the algae and find it necessary to replace the inappropriate term Phycomycetes with the new term — L e u c o p h y c e a e [203]. These Leucophyceae have no relationship to fungi.

Another conclusion of my theory is the dissolution of the kingdom Protista — these zoophytes of the 19th century that are supposed to represent a kingdom of transitional organisms that had not yet differentiated into true animals or true plants.

[202] From this point of view it would be extremely interesting to study a number of fungi which are usually classified as ascomycetes: *Ascoidea, Dipodascus, Taphridium, Protomyces, Monascus*. It would be particularly important to clarify the following points: If the cell wall consists of cellulose or of chitinous substance, whether the protoplasm has amoebiod movement, similar to that of the Leucophyceae, whether epiplasm remains in the sporangia. It is also necessary to determine the sensitivity of these organisms to temperature and toxins, and whether they are capable of assimilating nitrogen and carbohydrates from inorganic substances. It may be that all these are not fungi but Leucophyceae.

[203] Some authors are already inclined to this point of view, although the majority of botanists (B r e f e l d , B l a k m a n n , H a r p e r [1900], B a r k e r [1903], H. F i s c h e r [1904], D a n g e a r d [1898–1905]) continue to derive the fungi from the phycomycetes.

[367] In reality there are no such transitional organisms because there is no transition between symbiosis and non-symbiosis. Either a symbiosis with cyanobacteria is present — in which case we are dealing with a true plant, or there is no symbiosis — in which case we are dealing with a true animal[204] — with the exception, of course, that a given organism devoid of plastids originated from a fully developed plant. Every organism is therefore either an animal, a plant or a mykoid.

All of the foregoing is summarized in the accompanying figure.

In the figure, the mykoplasma is represented by thin lines, the amoeboplasma by thick lines, and the cyanobacteria or plastids by dotted lines.

From the figure it is evident that the organic world is composed of two phyla *[zwei Stämme]*, which descend from two independent roots. The phylum on the left is composed of the urbacteria — biococci, it is the kingdom of the mykoids which gives rise to two great groups of fungi — *Basidiomycetes* (fruiting fungi) and *Ascomycetes* (hyphal fungi), and a side branch, the cyanobacteria. This phylum appeared before the other. Later, the second plasma, the amoeboplasma, arose in the form of Monera. The micrococci, which penetrated into the Monera several times (symbiosis I), gave rise to the cell nucleus and consequently to the cell, thereby giving rise to the simple animals — the amoebae and flagellates. The latter were invaded by the cyanobacteria (symbiosis II), forging the plant kingdom. A side branch of the latter (on the left) comprise the Leucophyceae. The remaining amoebae and infusoria evolved into the animal kingdom.

[End Part IV, vol. 30, No. 11, June 1, page 367]

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[204] The same applies to plants as it does to lichens, which themselves represent a symbiosis of fungi and algae. Either the symbiosis is present, and they are lichens, or the symbiosis is not present, and they are fungi; there are no transitional forms nor can they exist.

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