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

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ABSTRACT

In 1910, the Russian biologist Konstantin Sergejewitch Mereschkowsky (Константин Сергеевич Мережковский, in standard transliterations also written as Konstantin Sergeevíc Mérežkovskij and Konstantin Sergejevich Merézhkovsky) 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;
- Nitrogen fixation: atmospheric nitrogen fixation ancient, nitrogenuous fixation derived;
- Plant symbiosis: plant symbiosis ancient, animals in symbiosis 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...
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 Occam’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 mykobacterioplasma. 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 N₂ as the sole nitrogen source. He viewed the ability to fix N₂ as extremely ancient, just as the ability to fix CO₂ was ancient in his view, and the first organisms he inferred (micrococcii) 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 (N₂ 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 N₂) 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 mukes, fungi) derived from mykoplasma, rather than bacteria derived from bacterioplasma.

Using physiological traits, the 1910 paper fleshes out the foundation for his initial expose (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 CO₂ exhaled during respiration did not derive from digested 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 until about 1960. Protoplast 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 mechanismists (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 (protops, 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 Protoplasmonic 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, protein, 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 biochemical 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 split 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 (mykplasma) 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 Protoplasmarten” 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 hped upon 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 irrebutably 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).

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 bi-layers 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 reincarnations of Mereșchowsky’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 3-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 Hans Ris, who had the basic idea so right that he even predicted gene transfer from organelles to the nucleus (Ris, 1925, 1927). Margulis (Sagan) wrote on the second page of her 1967 paper “… these ideas are not new …”, mentioning Mereșchowsky 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 Faintizyn (1907) and Mereșchowsky (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 fail 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 Sapropelgiales as members of the fungal kingdom. Instead, he identified these siphonaceous filaments as plants that have lost their plastids and classified the Oomycota among bacteria and the term chromatophores with plastids. Our translators harbour a highly reduced plastid genome (McFadden et al., 1996).

Mereschkowsky did not classify the Saprolegniales as members of the Oomycota. Instead, 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 halobacteria (simple forms), trichobacteria (filaments), actinobacteria (endospore 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 amoeboид Moneran. According to current data, the host was an archaeon (Imachi et al., 2020), not an amoeboид 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: Meroschowsky C (1918) Theorie der zwei Plasmaarten als Grundlage der Symbiogenese, 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 199, 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. Meroschowsky.

Contents. Preface. – I. Two lineages of plasma. II. Respiration. – III. Relationship to temperature. – IV. The synthesis of proteins. a) The Bacteria. b) The Fungi. c) The Cyanobacteria. d) Plastids. – V. Motion. – VI. Chemical composition. – VII. The relationship to toxins and general robustness. VIII. The other differences. IX. Conclusions from the theory of two plasma lineages. [279]

Preface.

If a problem has not been recognized, it cannot be subjected to investigation. J. Reinke.

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 (Darwin, Haeckel, Nageli) 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 one1 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 the Theory of Symbiogenesis.

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 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. [Das gibt die allgemeine Uberzeugung, dass der Baum des Lebens ein einziger ist.] 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.

The concept of the uniformity of the organic realm has to be abandoned in favor of the idea of its duality.

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

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.

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1 Meroschowsky, C. Uber Natur und Ursprung der Chromatophoren im Pflanzenreich. Biol. Centralbl. Bd. XXX, 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 so 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. 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. It 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 | Amoeboid plasma |
|---------------|-----------------|
| Mycoplasma | Amoeplasma |
| 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 Amoeboplasma, 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 Mykoids—the term I use to collectively designate the bacteria, fungi, cyanobacteria, including the plastids living symbiotically within the amoeboplasm, as well as of certain components of the cell nucleus. This immobile 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 amoeplasma 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 Mykoplasm.

It is possible that I will be confronted with the criticism that I use a term previously introduced by Erksson to describe a specific hypothetical cytoplasm by which certain fungi (Uredinear) 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. As a

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1 Ward demonstrated that the bodies that Erksson considered to be the initial secretion of fungal cytoplasm appearing to merge with the plant cytoplasm reflect nothing other than basidios of the fungal hyphae extending into the host’s cytoplasm, which nourishes the fungus. Ward, Marshall, On the histology of Uredo dispersa (Erksson) 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. [Erksson responded kindly in September, Biol. Centralbl. 30:618-623 (1910).]

Let us now consider, in more depth, all six main differences between myxplasma 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. [238] According to Pfeffer 9, "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 10; such that "the interruption of respiration can be considered the unerring proof of death."

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 (spalling of gas), the term spalling of gas is an archaic term for fermentation, that is, by so-called intracellular respiration like in fruits and other plant organs 10, or in some parasitic worms 11, one cannot consider this kind of respiration to be anything other than pathological 12, because in the end any organism will inevitably die following absence of oxygen.

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

A miraculous exception to this rule are the bacteria — one of the members of the myxoids. 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 also 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 Schm idt and Weis 13 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 the rules that generally apply for all living organisms. Especially Bejierinck 14 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 conditions...
conditions', one then has good reason to assume that anaerobiosis was the first and most ancient [resilience, primordial] bacterial condition. Hence it follows that the archaebacteria 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 an anaerobic origin 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 anaerobes. 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, [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 mycoplasma 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 mycoplasma, which are conserved in the kingdom of fungi, such that we may eventually find the individual steps of respiration to reveal specific character in fungi which discriminate them from those of the amoeboplasma [287].

### III. Relationship to temperature.

Kühne [27] 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 plasma in relation to temperature. The contractile plasma coagulated already at 40 °C and disintegrated, the remaining part of the plasma at 45 °C. Max Schultz [28] found that plant cells withstand 47 °C, beyond that they died [29]. Since then many observations were made with respect to the resistance of various organisms to high temperatures which are summarized in tables of Fürth [30] and Davenport and Castle [31] which served as material for the following brief compilation.

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10 W. Willinsky, W., Arch. f. Hygiene. Vol. LIV, Issue 4, 1905.
11 The fungi, as we will see in the further course of our treatise of the physiology of symbiosis, 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 Basidiozym. In fact, if we look carefully at the way spores are formed in bacteria in general and in Bacillus subtilis in particular (Schaudinn, F.), Beiträge zur Kenntnis der Bakterien und verwandter Organismen. I. Bacillus subtilis n. sp., Arch. f. Protistenkunde. Vol. I, 1902, p. 360), we must conclude that this is a fundamental correspondence in the way spores are formed in both groups. With Bacillus subtilis, 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 spores of the fungus, 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 Actinomyces, 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 conidium, 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 protoplast, 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. Entwicklungsgesch. der Bakterien. Flora, Vol. 84, 1907, p. 240).
12 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 synthesise 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 amoeboplasma. 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 Börting's opinion (Bürting, K., Botan. Centralbl. 1905, p. 298), the ability of bacteria capable of fixing free nitrogen is always related to the ability to live in the absence of 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.
13 Kühne, Untersuchungen über das Protoplasma und die Kontraktibilität. Leipzig 1866.
14 Schultze, Max. Das Protoplasma der Rhizopoden und der Pilzenleistigen. Leipzig 1883.
15 According to Meyer 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 metazoa the maximum temperature usually resides between 36 and 40 °C, it nearly reaches 45 °C, so that the survival for the majority of life forms is excluded above 45 °C. — Meyer, I., Die Selbstinheitung des Heus. Jena 1907, p. 89.
16 Führer, Otto. Vergleichende chemische Physiologie der niederen Tiere. Jena 1903, p. 424.
17 Davenport und Castle, Studies in Morphogenesis. III. On the acclimatisation of organisms to higher temperatures. Arch. f. Entwicklungsmechanik, Vol. II, 1896, p. 227.
| Animals               | Maximum °C | Plants            | Optimum °C | Maximum °C |
|-----------------------|------------|-------------------|------------|------------|
| Astrospillum         | 40         | Macrococcus       | 20—25      | 33         |
| Amoeba               | 40—45      | Macrococcus       | —          | 50         |
| Actinopagys          | 42         | Spirogyra         | —          | 44         |
| Several flagellates  | 60         | Chlorophora       | —          | 45—60      |
| Carchesium           | 47         | Oedogonium        | —          | 44         |
| Sterile              | 44—50      | Ulothrix zonata   | 15         | 24         |
| Actinia              | 38         | Vaucheria repens  | 30         | —          |
| Ibeno ovinus         | 38         | Hydrurus fistulos | 10         | 16         |
| Cottus venustis      | 34         | Plant cells according to Schulte | — | 47—48 |
| Medusa               | 36—39      | Trichococcus vulgaris | 29 | 42 |
| Varia varia          | 30—40      | Sinapis alba      | 27         | 37         |
| Aplysia              | 33         | Acet platanoides  | 24         | 26         |
| Amoebid              | 30         | Phaeocystis silvestris | 27 | 34 |
| Urechis              | 39—41      | Phaeocystis multiseta | 34 | 46 |
| Vorticella sp.       | 30         | Zoa main          | 34         | 46         |
| Deltia sp.           | 27—30      | Cucumis pepo      | 34—46      | 46         |
| Cyclopodium          | 34         | Eldred candelospor | 42         | —          |
| Oxygenta              | 36         |                    |            |            |
| Gambiastis           | 36         |                    |            |            |
| Palaeon               | 26         |                    |            |            |
| Coelilia pacificus    | 40         |                    |            |            |
| Hippocampus          | 30         |                    |            |            |
| Frog                 | 44         |                    |            |            |
| Salamander           | 44         |                    |            |            |
| Dog                  | 44—45      |                    |            |            |

(To be continued.)

At this temperature death did not yet occur; the movement continued even at 42°C. [Hautpfleisch, J. Biotot., Vol. XXIV, 1892, p. 490], but how long the plant remained under the influence of this temperature is not listed.

The table on p. 288 demonstrates that in the vast majority of cases the amoeboplasms 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 mycoplasma at higher temperatures. Oscillaria 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 Hoeppe-Seyler (25), these algae were found alive at even higher temperatures. Hapalosiphon laminosa, another cyanobacteria, lived in water above

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20 Dallinger, W. H. On a series of experiments made to determine the thermal death point of known Monard germa, when the host is enduring in a fluid. Jour. 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 of the credibility of the facts cited by Dallinger. Doubts are mainly raised by the question what this author understands under the terms Monard Germa, that is, whether he really worked with amoeboplasms 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.

21 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. Jour. Royal Microsc. Soc. 1887, p. 185—199) on protozoa, the maximum temperature of which was 60°C, 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°C. — 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°C, then perhaps if he had applied the method of gradual habituation to bacteria or any organisms, 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.

22 Hoeppe-Seyler, Physiol. Chemie, 1. Berlin 1877.
90 °C (32). Went also states that “the highest temperature at which filamentous Myxophyceae [archaeal] (that is, cyanobacteria) are known to exist is 86 °C” (39) and adds “but unicellular algae have been observed by Brewer in California at 94.4 °C” (40).

These observations were frequently disputed (39), 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 (35): “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 kingdom Protista. 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 (41). 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 myxobacterial plasmas. — Occasionally bacteria may multiply even at 75 °C at which temperature any protein known to us will coagulate. Yet Miche (31) and Kaulinski (30) observed bacteria (Bacterium ludwigii, Bacillus calfactor, Bacillus licheniformis) 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 are 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 fermentation the temperature may increase dramatically, sometimes inside the intestine of humans and animals where, according to Robinowitsch (33), 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 thermostolerance 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. Eisenberg was convinced that it was not the spores of that bacterium which survived this high temperature. He obtained similar results for the oidia [archae for conidia or arthrospores] from a few cultures that resisted 98 °C for 15 minutes. Similar results he obtained from the soil bacterium Bacillus thuringiensis and two other bacteria: Bacillus megatherium and B. rumous lipasecificus.

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 by hot vapor and high pressure when autoclaved at the following temperatures dependent upon the duration of the steam treatment:

| Temperature | Duration |
|-------------|----------|
| 140 °C      | 3 hours  |
| 150 °C      | about one hour |

(293) That is, there are bacterial spores that can survive almost an hour at 150 °C and remain viable! Verworn (38) says: “To date we have no plausible explanation for this mysterious phenomenon. We can only suggest that 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 protoplast, but with the protecting properties of the unusual

32 Schmidt, A., W. Weis, F., Die Bakterien. Jena 1902, p. 144.
33 West, G. S., Some Algae from Hot Springs. Journal of Botany. July 1902, p. 241.
34 West, G. S., A Treatise [sic] on the Freshwater Algae, 1904, p. 367, in which Brewer’s paper is quoted. Brewer, W. H., American Journal of Science, Ser. 2, Vol. XL.
35 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 observation, as we see shortly, remove all doubts.
36 De Vries, J. H., Arch. Neerland. V, 1870, p. 385. The quotations are taken from Lowy (Vortrag über botan. Stammesgeschichte, p. 374).
37 It would be extremely interesting to conduct experiments on the resistance of plants (especially in lower plants) to high temperatures. If the plants are observed outside and inside the cells, it might be possible to show that the plants are able to withstand higher temperatures than the amoeboplasms using Ringelmann’s bacterial method to reveal the assimilation activity of plants.
38 Miche, H., Die Selbstzehrung des Heicus. Jena 1907.
39 Kaulinski, Zur Kenntnis der Bakterien der Thermalequellen. Hygicus. Rundschau Vol. 5, 1895, p. 885.
40 Recently it has been shown that Robinovitch’s opinion is unfounded, that is, that the presence of oxygen does not have the effect that Robinovitch ascribes to this factor.
41 Eisenberg, P., Über die Thermorezistenz der vegetativen Formen der eurischen Sporenbakterien. Centralbl. f. Bakteriologie (Abt. 3), Vol. XVII, 1908, p. 87.
42 Schmidt, A., W. Weis, F., Die Bakterien. Jena 1902, p. 155.
43 Lafer, F., Handbuch der technischen Morphologie. Vol. I, Jena 1905, p. 447.
44 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 they require critical reevaluation to such a degree that I will simply disregard them here.” Sachs, Z., Lehrbuch der Botanik. 3rd 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. 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.

![Table]

| At          | 100° only | after 16 hours |
|-------------|-----------|----------------|
| 100°-110°   |           |                |
| 110°        |           |                |
| 120°        |           |                |
| 125-130°    |           |                |
| 140°        |           |                |

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 Lewin has shown, the coagulation temperature rises considerably with decreasing water content of the protein.

Pfeffer, 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 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 amoeboid plasma and mykoplasm regarding their behaviour to temperature: whereas the mykoplasm is able to increase its density in a way that the amoeboplasm cannot, this character reflects important differences distinguishing the two plasma lineages. These capabilities allow the amoeboid 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.

Obviously, we are faced with a kind of protoplasm that is of a different nature from that of the amoeboplasm. Therefore, Pfeffer 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, do not contain such proteinaceous compounds coagulating already at lower temperatures.”

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 gradually led the mykoplasm, of which also the fungi are composed, to a more or less close similarity to the life properties of the amoeboplasm. Under the influence of their parasitic or saprophytic lifestyle, the plasma of fungi relinquished some of its robustness [hat sich verwässert] although the unmistakable imprint of the original rough, crude mykoplasm, the bacteria, from which the fungal plasma descended, can still be discerned.

Tiskinsky, 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 on damp millet seeds. Tiskinsky also found two species of Actinomycetes of which one grows very frequently in soil, dung, hay, straw, potatoes etc., which was named by her as Thermoactinomyces vulgaris; it grows heat (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 mykoplasm towards high temperatures when compared with a similar compilation shown in the table for amoeboplasm (see page 287 f.).

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66 Migula (see Lafi, 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.

67 Pfeffer, W., Pflanzenphysiologie, Vol. I, Leipzig 1897, p. 54.

68 In reality, as we have seen, there are spores that, when wet, can withstand a temperature of 135 °C for one hour.

69 Lewin, S., Über die Ursache der Widerstandsfähigkeit der Sperren gegen hohe Temperaturen. Arch. f. experim. Pathol. u. Pharmakol. Vol. 26, 1890, p. 351.

70 Pfeffer, W., Pflanzenphysiologie, Part II, Leipzig 1904, p. 294.

71 Cohn, F., Beiträge zur Biologie der Pflanzen, Vol. 2, 1887, p. 266.

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86 It is possible that both properties of mykoplasm—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 mykoplasm. I urge those who like to give to the essence of things a causal link to think up two micellar theories, one for amoeboplasm and the other for mykoplasm, which should be constructed in such a way that the latter would simultaneously explain the ability of mykoplasm to tolerate high temperatures and its inability to move.

87 Tiskinsky, J., Sur les Mycéliales thermophiles. Annales de l'Institut Pasteur. Vol. XII, 1899, p. 500 and the same: Sur les microbites thermophiles des sources thermales, L., p. 788.

88 Behrens, J., in Lafi, Handbuch der technischen Mykologie, Vol. I, Jena 1905, p. 449.
### Table of Maximum Temperatures in Myxoid Organisms

| Organism                                      | Optimum °C | Maximum °C | Observer       |
|-----------------------------------------------|------------|------------|----------------|
| Bacillus liliæmicis capsulatus                | —          | 80         | Kariánki       |
| Closetrichia                                  | 55         | 65         | Kedrier        |
| Bacillus No. 2                                | 53—56      | 70         | Tsülkinský     |
| Bacillus No. 3 et 4                           | 68—71      | 73(?        | Tsülkinský     |
| Mould on bread                                | 56         | 7          | Bethens        |
| Thermomonospora lanuginosa                   | 57—55      | 63         | Tsülkinský     |
| Thermoactinomyces vulgaris                    | 57         | 70         | Tsülkinský     |
| Whose spores tolerate 20 min in steam         | —          | 100        | Tsülkinský     |
| Streptococcus                                | 53         | 62         | Sarmé          |
| Bacillus ramosus                              | 25—28      | 38         | W. Ward        |
| Aspergillus fumigatus                         | 35—40      | 60         | Réno, Cohn     |
| Aspergillus lignieresi                        | —          | 58         | Constantin et Luzet |
| Aspergillus micro-virido-citrinus             | —          | 45         | Constantin et Luzet |
| Thermophilic bacteria                         | 60—70      | 75         | Globig, van Tsiglom, Sarmé |
| Thermoidium sulphureus                        | 35—45      | 53         | Miehe (Ber. d. d. bot. Ges. 1908) |
| Bacillus subtilis                             | —          | 50         | Breifeld, Schreiber |
| Bacillus, gradually accustomed to             | —          | 58         | Tsülkinský     |
| Bacillus subtilis, spores 25—30 min           | —          | 140        | A. Meyer       |
| Ustilago carbo, spores, dry                   | 104—120    | 104—120    | Hoffmann       |
| Ustilago aurodecum, alike                     | —          | 140        | Payen          |
| Oidium aurantiaclum, alike                    | —          | 127—132    | Pauleur        |
| Penicillium glaucum, alike                    | —          | 138        | Schacht        |
| Peziza rupida                                 | —          | 86—90      | De Vries       |
| Cynobacteria                                  | —          | 85         | Went           |
| Cyanobacteria                                 | —          | 94.5       | Breuer         |
| Hepalosiphon luminosus (Cyst.)                | —          | 90         | Schmidt & Weis, Bakt. 144 |
| Oscillatoria                                  | —          | 64.7       | Hoppe Sleyer   |
| Saccharomyces                                 | 28—34      | 34—40      | Pedersen, Hansen |
| Saccharomyces, dry                            | —          | 60—65      | Kayser         |
| Saccharomyces, dry, 15 min                    | —          | 115—120    | Mansaein       |
| Saccharomyces, dry, 15 min                    | —          | 75—80      | Zofp           |
| Thermosaccus aurantiacus                      | —          | 33         | Miehe          |
| Spores of bacteria, moist, 1 min              | —          | 140        | Christen       |
| Spores of bacteria, dead, 1 hour              | —          | 150        | Koch, Woffühigel |
| Bacterium ludwigii                            | 55—57      | 80         | Kariánki       |
| Bacillus caffaeur                             | —          | 65—70—80   | Miehe          |
| Bacillus anthracis, vegetative, State         | —          | 70         | Eisenberg      |
| of sporulation, 15 min                        | —          | 90         | Eisenberg      |
| Bacillus anthracis, allix, 5 min              | —          | 98         | Eisenberg      |
| Bacillus anthracis, allix, Oidium, 15 min     | —          | 70—98      | Eisenberg      |
| Bacillus megatherium, vegetative              | —          | 70—98      | Eisenberg      |
| Bacillus ramosus, vegetative                  | —          | 70—98      | Eisenberg      |

[295–296] 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 mykoplasm synthesizes proteins from simple inorganic substances — an ability that the amoeboplast lacks...
altogether. Therefore the latter can only exist and propagate if it is provided with proteins from outside.\(^{12}\)

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.\(^{13}\) The plastids, as already set forth elsewhere, are not germaine to the plant cell itself.\(^{14}\) — Indeed, there exist strong arguments not to interpret plastids as organs or organeloids of the cell that plant cells generate endogenously, but as specific organisms of the mykold 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.\(^{15}\)

\(^{12}\) 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 environment. 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."

\(^{13}\) 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.

\(^{14}\) Merechowski, C., Über Natur und Ursprung der Chromoplasten im Pflanzenreich. Biol. Centrbl. Vol. XXV, No. 18, 1905.

\(^{15}\) 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 Micrococcus racematus. Laurent, E., Recherches sur la valeur comparée des nitrites et des selles amonaces comme aliments de la levure de bière et de quelques autres plantes. Annales d. Institut Pasteur Vol. 3, 1899, p. 362) and a rather negative hint by Falk with regard to Sporodinae 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 Staphylococcus lactis (acronym) "cette mucidine" (I. c. p. 370). Accordingly, one can doubt whether he was really dealing with phycocyanins in this case.

\(^{16}\) Laurent, E., Comptes Rendus d. l. Acad. d. Sc. Paris, Vol. CXV, 1895. (ibid. Vol. CXVIII, 1894. — Archiv des Sciences biologiques de l'Institut de Médicine Experimentale, St. Petersburg, 1895, Vol. III, Issue 4. — Clastadium pasteurianum, seine Morphologie und seine Eigenschaften als Butterausferment. Centralbl. f Bakteriologie, Vol. 9, 1902, p. 3.

\(^{17}\) 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 of A. Koch (see Laffr., Handbuch der technischen Mikrologie, Vol. III, Jena 1904, p. 9) there is no reason for these doubts. — Benezek and Rumler believe that Azotobacter is not a bacterium at all, but belongs to the cyanobacteria and consider it a colourless form of Aphanoaeca.

\(^{18}\) Stocklowsky, J., Über die chemischen Verhältnisse der Assemblage des Eierkernes und die Kernen. Biochemische Zeitschrift, Vol. XVII, 1906, p. 144, cited after Czapek, I.C.
nitrate salts. Such bacteria are numerous\(^{2,3}\), 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, Thiorhizia 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\(^{52}\).

Čapek\(^{52}\) writes: “Surprisingly many microbes are able to utilize the simplest compounds of carbon chemistry and in this regard are no longer distinct from nitifying 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\(^{52}\) for nitifying 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 ammonium to nitrite or the oxidation of nitrite to nitrate.

According to Kaserner\(^{53}\), the soil bacterium Bacillus pasteurii reduces CO\(_2\) to formaldehyde and subsequently to more complex compounds by oxidizing hydrogen. Another bacterium detected by Beijerinck and Delden\(^{54}\), Bacillus oligoeleuthrophilus, is reported by the same author to initially reduce CO\(_2\) to CO\(_3\), from which it then synthesizes its organic compounds without releasing oxygen. The same ability to assimilate CO\(_2\) without producing oxygen was reported by Nikolski\(^{55}\) for colorless bacteria. – The same is also true for marine sulfur bacteria (thiobacteria) according to Nathanson\(^{56}\). Beijerinck\(^{57}\) 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. [310] The energy required for this chemosynthesis comes from oxidizing sulfur. The former oxidizes carbon disulfide \([^\text{Fe(II)}\text{S}_2\text{O}_3\text{}}\) to sulfur or oxidizes Na\(_2\)S\(_2\)O\(_3\) or Na\(_3\)S\(_2\)O\(_6\) to Na\(_2\)SO\(_4\) 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:

\[
6 \text{KNO}_3 + 5 S + 2 \text{CaCO}_3 = 3 \text{K}_2\text{SO}_4 + 2 \text{Ca} \text{S}_2\text{O}_3 + 2 \text{CO}_2 + 3 \text{N}_2
\]

If one provides these bacteria with sulfur 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\(^{59}\).

The Fungi.

Not a single animal can live from carbohydrates (sugar, starch) and fat alone without a supply of nitrogen containing substances because the amnoplasma of animals is incapable of synthesizing nitrogen containing compounds like proteins from inorganic substances\(^{51}\). Fungi, however, which consist of mycoplasmata, 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\(^{52}\) 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 oxidation of carbohydrates\(^{53}\). [No fungi are autotrophic \(^{52}\).] 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 (\textit{als Quelle der Kohlenstoffe 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\(^{53}\).

But fungi are also able to assimilate free nitrogen from the air in the same way as bacteria\(^{53}\). Of this there can be almost 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).

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

Pfeffer, W., Pflanzenphysiologie, Vol. 1, 2. Edition, 1904.

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

Lafar, Fr., Handbuch der technischen Mykologie, Vol. 1, Jena 1904, p. 402.

The view of Frank and some others that green plants can assimilate nitrogen from the atmosphere can now be regarded as refined 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:

51. Lafar, Fr., Handbuch der technischen Mykologie, Vol. I, Jena 1904, p. 412.
52. Lafar, Fr., ibidem, p. 410.
53. Czapek, F., Die Ernährungphysiologie der Pflanzen seit 1896, Progressus in botanica, Vol. 1, Jena 1907, p. 479.
54. Winogradsky, S., l.c.
55. Kaserner, H., Die Oxidation des Wasersstoffes durch Mikroorganismen, Centralbl. f. Bakteriol., (Er. Department), Vol. XVI, 1908, p. 681.
56. v. Delden, A., Centralbl. f. Bakteriol., (Ser. II), Vol. II, 1903, p. 81.
57. Nikolski, M., Ein Beitrag zur Kenntnis wasserstoffoxydierender Mikroorganismen, Bulletin d’Acad. d. Cracovie, Classe des sc. math. et nat. 1906, p. 911.
58. Nathrath, H., Uber eine neue Gruppe von Schwefelbakterien und ihren Stoffwechsel. Mitt. a. d. anorg. Statist zu Nepal, Vol. 15, 1902, p. 655.
59. B e i j e r i n c k, M., Observationes de reductione producta per les microbes. Archives Néerlandaises des sc. ex. et nat. Ser. II, Vol. IX, 1904, p. 131. – Ref. in W. Roux, Centralblatt, 1904, p. 298. – See also Centralblatt f. Bakteriol., Vol. XXI, 1904, p. 693.
60. Lafar, Fr., Handbuch der technischen Mykologie, Vol. I, Jena 1904, p. 418. This ability regarding the purple bacteria is based on their use of CO\(_2\) and the light energy from the sun for their growth.
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. [No fungi are dioecious.]

Especially interesting in this respect are experiments carried out by Ternietz using a fungus that grows on the roots of various Ericaceae. Ternietz cultivated this fungus axenically in a nitrogen deficient medium. In this medium the fungus grew rapidly, and Ternietz, through exact analyses, was able to demonstrate the increase in nitrogen content, which only could have its source in the atmospheric nitrogen. [305] Ternietz had no doubt that she was dealing with a true fungus, as demonstrated by the mycelium, which was divided by septate 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 endophytic mycorrhiza, that these fungi assimilate free nitrogen from the air to synthesize their own protein. [306] [probably contaminating N₂ fixing bacteria, perhaps actinomycetes like Frankia in the case of the mycorrhiza.]

[End Part II, vol. 30, No. 9, May 1, page 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, 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 with Nostoc demonstrating that this cyanobacterium is able to assimilate inorganic nitrogen as a nitrogen source as it grows well under 0.1% KNO₃.

Yet there are also reasons to believe that cyanobacteria, similar to bacteria and fungi, may assimilate free nitrogen from the air. [323] 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 these structures. Of course, this explanation is not correct, but the benefit for the plant appears to be evident. Koch [324] summaries 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 CO₂ 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. [325] 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 protein increases simultaneously. Finally, as Sachs 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 concentrations in the cells of leaves. If proteins are also formed in the plant roots and apparently only from

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

However, it must be kept in mind that Müller recently (Berichte d. deutsch. botan. Gesellschaft, 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.

Ternietz, Ch, Assimilation des atmosphärischen Stickstoffs durch einen totholzigen Pilz. Berichte d. deutsch. botan. Gesellschaft, 1904, Vol. 22, p. 267.

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

Kühl, C., Über die Organisation und Physiologie der Cyanophyceanzelle. 1903.

Loew, O. Verhalten milderer Pilze gegen anorganische Stickstoffverbindungen. Biol. Centralbl., Vol. X, 1899, p. 591. [footnote missing. belongs at that spot]

See the experiments of Bouilhac and Giustiniani (L’année biologique, 1905, p. 204) which prove that Nostoc and Anabaena can develop vigorously in a medium that is completely nitrogen-free, presumably they drew 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 Anabaena and Anabaena, two cyanobacteria, are able to fix atmospheric nitrogen (Beijerinck, Centralbl. f. Botanik, Vol. VII, 1901, p. 562, Bus Crapek (Buchner, d. Pflanzen, Vol. II, p. 230) also counts these experiments, which were not supported by the necessary analyses, as insufficiently convincing.

Koch, A., Der Kreislauf des Stickstoffes, in L. far, Handbuch d. techn. Mykologie, Vol. III, Jena 1904, p. 64.

Sachs, J. Vorlesungen über Pflanzenphysiologie, Leipzig 1882. See also Pfeffer, W., Pflanzenphysiologie, Leipzig, Vol. I, 1897, p. 402, and Crapek, F., Biochem. d. Pflanzen, Vol. II, Jena 1905, p. 211.
amides, the roots also have plastids. If proteins are also formed in the plant roots and apparently only from amides, the roots also have plastids.

Benecke, W., Über farbhlose Diatomeen der Kieler Fährinde. Pringsheim's Jahrb. f. wissensch. Botanik, Vol. 35, 1900, p. 533.

Kas ten, G. Über farbhlose Diatomeen. Flora oder allgem. botan. Ztg., Vol. 89, 1901, p. 462.

This is just as clear and unquestionable as the following attempt would be: one has 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 enveloped 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 the same degree correct and unassailable as the conclusion from Kasten'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.

Unfortunately, Kasten did not attempt to cultivate Nitzschia purpurea in a solution containing inorganic salts and some hydrocarbon (e.g., Isobutenol), 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 proteins 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 myoplasm 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, such as Siphonales and Siphonocladales; for instance, in the macroscopic multicellular alga Caulerpa, the interior is traversed by protoplasmic strands in which the protoplasm 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 spermatozoa exhibit amoeboid-like movements in that they crawl upon the oogonia like a small amoeba. In the green algae 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 protoplasm are found, circular movements as in Valonia spiralis and Hydrocharis, or streaming movements as in the staminal hairs of Tragopogon virginianus, Lamium, pumpkin etc. are widespread[69].

One has to keep in mind that the movement of the protoplasm in plant cells is of two types: [327] Primary or continuous if the streaming is continuously observed in undamaged cells and secondary 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[1].

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 myxoids.

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 amoeboplasm, as work by Tavernier[70] 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 protoplasm shifts into one direction or the other, similar to low tide and high tide[71].

[328] The cyanobacteria likewise do not exhibit any movements of their plasma[70], the same applies for plastids[71]. 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 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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[69] See with regard to this question Wigand, Botan. Hefte, Forsch. a. d. botan. Gärten zu Marburg 1: Issas, 1885, where all known cases of movement of plasma in plant cells are compiled and classified. The view expressed by Keller that all movements of the plasma in plant cells are secondary movements, that is, are caused by tissue rupture and injury, apparently others as well (Hauptfleisch). 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.

[70] Hauptfleisch states: The flow of plasma is therefore present in all tissue fibers, it is not absent in any of them (Hauptfleisch, P., Untersuchungen über die Strömung des Protoplasten in beblätterten Zellen, Pflügers. J. 193, 1932, p. 18). [Footnote 10] missing in the text, probably belongs at the position indicated.

[71] Janett, Chi, Protoplasmabewegung und Fruchtäpfelspulung bei Acetabularia cornea Pers. Pflüg. J. 193, 1932, p. 273. Wronin observed similar movements in another acetabularine (Acolochus pulcherrimus) (Wronin, M., Beiträge zur Morphologie und Physiologie der Pflanzen, II. Serie). Arthur observed similar movements of passive character in Sporopoleus virginiensis (Arthur, J., Annals of Botany, Vol. VI, 1897).

[72] 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 Hohnmann. "The plasma of the spores and of the germination tube is contractile and motile like that of animal sarcod (sarcod 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 form of the tube that it had previously occupied (for an archaical expression). The movement is to be described as streaming." (Hohnmann, H., Untersuchungen über die Keimung der Pilzsporen, Pflügers. J. 193, 1932, p. 38). 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".

[73] In some filamentous cyanobacteria, e.g. Oscillatoria, Regelettana and Sporochaete (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 mucous on the surface of the filament; in addition, a male-like movement is observed, the cause of which remains completely unknown.

[74] 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, Senn'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 rhizopoda, heliozoa or radiolaria. 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[89] has proven that the flagella of the water fern spermatozoids terminate at densely staining bodies which Weber initially named blepharoplasts and which according to Belajeff may be derived from centrosomes. Ikken[90] confirmed this view by demonstrating it for cycales 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. 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, salmon, butterfly, Heliol originates from the centrosome (more correctly the centre),[100] that furthermore the axis of the pseudopodia in the protozoans Acanthocystis, Radioloplospora, and Actinosphaerium originate from the intensely staining nucleus, that in Campylocentrum minus 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 amoecoplasm and the mykoplasm.

VI. Chemical composition.

A remarkable difference between the mykoplasm and the amoecoplasm is also seen in their chemical composition. [331] — In this respect, however, we are faced with insoluble 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 Reink[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 mykoplasm appears to be enriched in phosphorus compared to that of the amoecoplasm. Hints come from facts obtained through the analyses of ashes of both animals and plants, which in great number are compiled and published in Wolf’s “Analyses of Ashes”[106].

Let us consider especially the data from fungi. From these we see that the P/O ratio is highly related to that of plants.

89 Gurtitsch, A., Morphologie und Biologie der Zelle. Jena 1904, p. 38 ff.
90 Belajeff, W., Über die Centrosome in den spermatozogenen Zellen. Ber. d. deutsch. botan. Gesellsch., Vol. 17, 1899.
91 Ikken, S., Die Spermatogonien von Marchantia polymorpha. Beiträge zum botan. Centralbl., Vol. XV, 1903. See also: Die Blepharoplasten im Pflanzenreich. Biolog.Centralh., Vol. XXIV, 1905. — The presence of centrosomes in liver masses has been denied by various observers (Miyake, Bissoyan and others), but since v. Leeuwen-Reijnwann (v. Leeuwen-Reijnwann, W. et J., Über die Spermatogonien der Moose. Ber. d. deutsch. botan. Gesellsch., Vol. XXV, 1908, p. 301) has recently reconfirmed their presence in Pogonilla, Pellia und Mnium with a certainty that leaves nothing to be desired, one has no reason to doubt this fact.
92 Jahn, E., Myxomycetenstudien. Ber. d. deutsch. botan. Gesellsch., Vol. 22, 1904, p. 84.
93 Hääcker, V., Praxishandbuk der Zellensubs und Bedeutungslehre. Jena 1899.
94 Gurtitsch, A., Morphologie und Biologie der Zelle. Jena 1904, p. 45.
95 Gurtitsch, A., l.c., p. 64, Fig. 30, p. 93, Fig. 43.
96 There are quite a few 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 epithelium of the Lammelbranchiata (Wallengren, H., Zur Kenntnis der Filamentzellen, Zeitschr. f. allg. Physiologie, Vol. V, 1905, p. 357). But in the given case, considering the extreme small size of centrioles and their inconsistency with regard to their stainability, the positive indications carry more weight than do the negative ones.
97 Fischer, A., Vorlesung über Bakterien. Jena 1903.
98 Reink, J., Ber. d. deutsch. botan. Gesellsch., 1904, p. 100.
99 Wolf, E., Acharnolysis von landwirtschaftlichen Produkten, Vol. I, 1871. — Vol. II, 1889. — See also König, Chemie der menschlichen Nahrung- und Genussmittel. 3. Edition, 1889. — Liebig, J., Chemie in ihrer Anwendung auf Landwirtschaft und Pflanzenernährung. 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:

| Plant | Fungi |
|-------|-------|
| Fucus vesiculosus (8) | Sphaecilla segetum |
| " serratus (3) | Dito on rye |
| " nodosus | " " " |
| Laminaria digitata (6) | " barley |
| Laminaria saccharina (3) | " smooth brome |
| Sargassum vulgare (3) | Cryptococcus femorment |
| Polysiphonia elongata | Dito, bottom yeast |
| Delmerta saucuse (2) | " wheat beer yeast |
| Ceramium rubrum | Tuber cibarium |
| Enteromorpha intestinalis | Helvelia esculent |
| Ulva lactuca | Mortella esculent |
| Algae in general (23) | " conica |
| Sphaegmum cuspidatum | Agaricus campestris |
| " Foretia | Bolletus, bich polyspora |
| " Hymenoblasta | Yeast |
| " Splendens | " |
| " Trigetum | " |
| Sphaegmum species | " |
| " near Berlin | " |
| Ascidiu felix femina | " |
| " mas | " |
| " leaves | " |
| Asplenium trichomanes | " |
| Osmunda spicata | Saccorhyzos mycosystems |
| Pteria aquilina | " cerovula |
| Male fern (9) | " |
| Lycepodium (6) | Boletus edulis |
| Fri, branches and needles | " semillatus |
| Spruce needles (8) | " semilucus |
| Oak (38) | Claviceps purpurea |
| Hay (106) | " Agaricus wantculiens |
| Grasses (107) | Claria flava |
| Clover flowers (113) | Sclerotinia libertina |
| Turnip (49) | Mutterknoll |
| Tobacco leaves (63) | Chantelleas |
| Spinach (2) | " Truffle |

According to Zopf[111] the ash of fungi contains on average 40% phosphoric acid, unknown from any group of organisms belonging to the algaeooids. 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 that “the supernormal high content of phosphoric acid in the ash of most fungi and bacteria”[114]. According to the calculations of Köppen[115] the content of phosphoric acid in the ash of Bacillus prodigious and B. cereus 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 protoplasm, 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

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110 W olff, I.c., Vol. I, p. 154 and Vol. II, p. 110. — It is interesting to note that lichens, which consist of mykoids (fungi) and algaeooids (algae), already have a much lower phosphorus concentration (W olff, I.c., p. 135).

111 F ischer, H., Die chemischen Bestandteile der Schimmeyzenen und der Lymphocyten, in L a f a r, Handbuch der technischen Mykologie, Vol. I, Jena 1904, p. 235.

112 S chmidt, J and W eis, F., Die Bakterien, Jena 1902, p. 107.

113 F ischer, H., I.c., p. 224.

114 I.c., p. 225.

115 W olff, E., Erscheinungen. 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 [10], which in the animal kingdom are caseins, in the plant kingdom phyto globulines [19].

|          | \( P\text{O}_5 \) | \( P\text{O}_5 \) |
|----------|----------------|-----------------|
| Human, muscles | 37.5 | 56.8 |
| Chicken | 36.5 | 39.5 |
| Calf | 36.3 | 39.9 |
| Eggs | 38.0 | 34.5 |

Regrettably, an analysis of the ash of cyanobacteria, as far as I know, has not been performed to date. Undoubtedly those mykoids will also possess a percentage of phosphorus not even less than that of fungi and bacteria [10].

The nuclei, however, which according to my theory primarily consist of mykoplasmata [10], are rich in phosphorus as is long-standing 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 mykoplasmata and the amoeboplasmata. We saw above that the mykoplasmata 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 amoeboplasmata (cytoplasm), apart from the chromidia (chromatin bodies) which, of course, come from the nuclei as Digby [21] has shown.

| Bacillus megaterium | Nitrogen | Phosphorus | Sulphur |
|---------------------|----------|------------|---------|
| " anthracis         | 16.32    | 1.85       | 2.10    |
| Aspergillus niger I | 15.66—15.74 | 2.16—2.25 | 1.95    |
| " II                | 15.19    | 0.84       | 1.12—1.21 |
| Bileteus edulis (cap) | 15.64—15.84 | 1.08   | 2.14    |
| Claviceps purpurea (sclerotia) | 16.62—16.23 | 0.75 | 1.77 |

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

\[10\] Cohnheim, O., Chemie der Eiwellk ö pfer, 2. Edition, Braunschweig 1904.

\[21\] 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 mykoplasmata.

\[23\] Recently, Stoklasa, Brdlik and Ernest have convincingly demonstrated that chlorophyll also contains a fairly large amount of phosphorus (Stoklasa, J., Brdlik, W. and Ernest, A., Zur Frage des Phosphorgehaltes des Chlorophylls. Ber. d. deutsch. bot. Gesellschaft, Vol. XXVII, 1909, p. 10). The negation of this fact by Willisslitter is apparently a mistake.

\[21\] Digby, L., Observations on “Chromatin bodies” and their relation to the nuclei in Galtonia candicans, Annals of Botany, Vol. XXIII, 1909, p. 491.

\[22\] Fischer, H., Die chemischen Bestandteile der Schizontmyceten und der Euryzoten, in L d. Handb. d. techn. Mykologie, Vol. 1, 1904, p. 245, where the literature on this subject is also compiled.

\[23\] Iwanoff, K.S., Hofmeister’s Beiträge z. chem. Physiol. u. Pharmacol., Vol. 1, 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 myxoids. The content of nitrogen containing substances of these species is as follows:

| Amides and peptones | Albumin | Nuclein |
|---------------------|---------|---------|
| In brewer’s yeast   | 16.11%  | 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 cytoplasm 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 mykoplasm is especially rich in nucleoprotein comes from a comparison of digestible and non-digestible proteins of fungi which entirely consist of mykoplasm (128) and that of plants (129) where the mykoplasm of the nucleus and that of the plastids appears as strongly diluted by the amoeboplasm, 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   |                               |
| Agaricus, porratus, 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 delicatissimus  | 6.8                          | 35.8              | Peanut cake                   | 0.345 —       |
| " terminus                | 11.8                         | 40.0              | Copa cake                     | 0.254 —       |
| Camellus 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 —       |
| Polyergus ovatus          | 6.3                          | 40.6              | Coconut cake                  | 3.549 —       |
| " hippocaricumen         | 5.0                          | 29.8              | Rapeseed cake                 | 5.443 —       |
| " repens                  | 9.3                          | 44.0              | Peasat                        | 8.132 —       |
| Sparrassis crispa         | 6.8                          | 37.4              | Lupin                         | 7.839 —       |
| Morchella esculenta       | 2.5                          | 38.1              | Mali sprouts                  | 4.167 —       |
| Lycoperdon bovista        | 5.2                          | 22.5              | Vegetable ivory               | 0.619 —       |

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., Zeitgesch. 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, citad from Laffer, Handb. d. inchi. Mykot., Vol. 1, p. 298.
126 Fischer, A., Die Zellen der Cyanophyceen. Botan. Ztg., Series 1, 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 mycoplasma (fungi) contain on average 35% insoluble proteins, [337] whereas in those organisms in which the mycoplasma 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 mycoplasma organisms and the nuclei of the amoeboid organisms are rich in nucleoproteins. But does the amoeboid nucleus contain it? Let us see what the experts say.

Verworn [320] states "It turns out that the nucleus primarily harbours the phosphorases containing compounds of the proteins and especially nucleic acids, which within the protoplasm appear to be altogether absent." Gurwitsch [315] 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 nucleoalbumins and therefore not representing real nucleic acids or xanthine bases containing bodies, are found inside the cytoplasm, according to numerous investigations". [338] Therefore, according to numerous chemical investigations, one encounters true nuclei (that is, nucleoproteins), exclusively among the mycoplasma, that is, inside the nucleus, in bacteria, fungi and cyanobacteria [339]. In typical amoeboid plasmas, 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 mycoplasma with their presence in the amoeboid plasmas (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 mycoplasma 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 [340]. 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 mycoplasma, 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 mycoplasma, especially as it is found in mycoplasma, although one encounters it occasionally in animals, too. This is glycerol. Errera [342] was the first to state that starch and sugar, acting as reserve substances in plants, is replaced by glycerol in fungi. Glycerol and similar substances have also been found more than once in bacteria, for example in Granulobacter polymyxa [342], in Asotobacter, and in cyanobacteria, respectively [342]. Further evidence for the different chemical composition of the two plasma lineages is found in the differences of the initial assimilation products among mycoplasma and amoeboids. In all green plants saccharose is widespread. It represents, as many physiologists like Brown and Morris suggest, the initial photosynthetic product following assimilation of CO₂. In all parts of green plants exists an enzyme called invertin 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) [342], and the enzyme invertin is replaced by a different enzyme — trehalase [342].

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 phosphorases (P₂O₅) content in the ash of a) cyanobacteria, b) bacteria, c) pure amoeboid cytoplasm without nuclei [343], d) purified nuclei without traces of cytoplasm.

2. To determine the phosphorases (P₂O₅) 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 phosphorases in the muscle ash.

4. To elucidate microchemically the composition of plasmas a) in relation to phosphorases 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 [342].
VII. The relationship to toxins and general robustness.

The robustness of the mycoplasma 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 amoeboplasmas, which succumb 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 Marré, 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 Marré. 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 katérobs (which are not considered here). The oligosaprobels require rather clean water, followed by the mesosaprobels and finally the polysaprobels, 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, whereas the number of mykoids gradually increases, thereby indicating that mykoids are more robust than amoeboids.

| General number of | Oligosaprobels (least fouled water) | Mesosaprobels | Polysaprobels (most fouled water) |
|-------------------|-------------------------------------|--------------|----------------------------------|
| Mykoids           | 27 i.e. 13%                         | 19 i.e. 9.5% |                                  |
| 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.** Similarly, Schmidt and Weis 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 who investigated the effect of mineral poisons on diatoms found that the following negligible doses of different toxic substances are lethal:

|                    | Still alive | Dying on |
|--------------------|-------------|----------|
|                    | exposure    | exposure  |
| Mercury chloride   | 1/10 000    | 1/10 000 |
| Copper sulfate     | 1/15 000    | 1/15 000 |
| Zinc sulfite       | 1/25 000    | 1/25 000 |
| Arsenic acid       | —           | 1/25 000 |

According to Davenport and Nealy even a solution of 0.0001% mercuric chloride kills some infusoria (Seton), but a 0.001% solution will kill them quickly. For higher animals (according to Bähring) 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. Kossjakow succeeded to get bacteria gradually used to even higher doses of poison as shown in the following table:

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**Kolkwitz, R. and Marré, M., Ökologie der pflanzlichen Saprobiens. Ber. d. deutsch. botan. Gesellschaft, Vol. XXVI, 1908, p. 505.

**K. V. Kowallik and W. F. Martin

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nuclei using existing methods (by shaking). Some data already exist concerning the amount of nucleic proteins in the cell nuclei (Kasell). Kolkwitz, R. and Marré, M., Ökologie der pflanzlichen Saprobiens. Ber. d. deutsch. botan. Gesellschaft, Vol. XXVI, 1908, p. 505.

Weber, Text-Book of Fungi, London, 1996, p. 127.

Schmidt, Johns and Weis, Fr., Die Bakterien, 1902, p. 104.

Just's Jahresbericht für 1892, p. 175.

Davenport, C. B. and Nealy, H. V., Acclimatisation of Organisms to poisonous Chemical Substances. Arch. f. Entwicklungsgesch. d. Organismen, Vol. II, 1896, p. 570. — Also according to Brokorn, Th., Arch. f. Physiol., Vol. CX, 1905, p. 203.

Schmidt, Johns and Weis, Fr., Die Bakterien, 1902, p. 171.
Although the amoeboplasma already dies at 0.0001% mercuric chloride, Bacillus subtilis withstands 0.01%. This means that the mycoplasma is 100-fold more resistant than the amoeboplasma. Bacillus subtilis 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. Actinomycetes odorifer withstands the following unbelievably high toxic concentrations\(^{145}\):

|          | % Borax | % Boric acid | % HgCl₂ |
|----------|---------|--------------|---------|
| range    | range   | range        |         |
| Bacillus subtilis | 11–18   | 9–11  | 0.07–0.10 |
| Bacterium antrachis | 4–7     | 6–8   | 0.05–0.07 |
| Bacillus (Thiochrysalis) tenuis | 16–21   | 9–11  | 0.16–0.17 |

And while the amoeboplasma dies already at 0.0001% mercuric chloride, Actinomycetes tolerates up to 0.01% of the poison, indicating that the mycoplasma is 100-fold more resistant than the amoeboplasma. If one believes Johan-Olsson\(^{146}\), Aspergillus niger even tolerates 1% mercuric chloride solution.

Similar results for the mycoplasma were obtained from another toxic substance called lapis. According to Bokorny\(^{147}\), the nematode N. bancrofti has 0.001% AgNO₃, while Actinomycetes 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\(^{148}\) 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"\(^{147}\).

The same results were noticed for fungi. Hoffmann\(^{149}\) reports that Schmitz observed spores of Penicillium expansum 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\(^{150}\). — By contrast, many bacteria live and propagate normally in 10% salt solution, in which they continue to secrete their typical enzymes\(^{151}\). 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\(^{152}\).

But that is not all. Lewadowsky\(^{153}\) 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\(^{154}\), without losing their viability.

Bacterial spores are even more resistant: those of Bacillus antrachis are able to survive in concentrated NaCl solutions for months, those of the diphtheria agent for three weeks\(^{155}\).

Bacteria are even able to live in herring brine, though they do not multiply\(^{156}\).

Apparently the mycoplasma 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 CuSO₄. Diatoms, for instance, as we have seen in the beginning of this chapter, already die at 1/10,000 of this salt, whereas according to Nägeli\(^{161}\) Spirulina and some other algae are even more sensitive to this poison and do suffer in solutions reported similar for Pleurococcus, whereas Spirulina and Vaucheria were less robust. A Richter succeeded in growing different freshwater green algae in highly concentrated salt solutions\(^{162}\). But here Oltmanns adds: "From the experiments of Richter and Drewitz it appears that the algae do not permanently tolerate high salt concentrations."

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

Fischer, A., Botan. Zeit. 1905, p. 104.

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Freitag, C., Zeitschr. f. Hygiene, Vol. XL, p. 50, from Czapek, Biochemie der Pilze, Vol. II, p. 800.

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Oltmanns, Fr., Morphologie und Biologie der Algen, Vol. II, Jena 1905, p. 184. — See also: Nägeli, Olygodanym. Erscheinungen in lebenden Zellen. 1893.
containing one part CuSO₄ in 50 million parts of water, according to Bohorny (143) even in dilutions of one to one hundred million.

Now we will see how fungi respond to this poison. Bohorny (143) states “Some fungi are relatively insensitive against CuSO₄, contrary to algae and infaustia which become easily damaged.” And De Barry (144) states “If we have investigated thallus of Penicillium glaucum a foot in length that have formed on the surface of CuSO₄ solutions used for galvanoplastic purposes”, similar to Berkely (145) who found this fungus upon solutions of ferric sulfate. Hoffmann (146) observed equal thalli of Penicillium glaucum in rich spore formation upon the surface of saturated arsenic acid. This was also found by Jaeger (147). Palis (148), who carried out many experiments investigating the resistance of moulds against CuSO₄, reported that Penicillium glaucum is remarkably resistant in this respect. He also recalls the “rather low resistance of Mucor in general” (145) and of the impedig 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 phycocyte, (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 HS and CO₂. — Bacteria (for example Beggiatoa) and cyanobacteria incorporate H₂S, which for animals and plants is highly toxic. Mucor (amoeboid) already suffers at 33% CO₂, for the fungus Penicillium only levels above 80% cause toxic effects. Many bacteria, however, live in pure CO₂ as well as do in air.

Numerous experiments on the effect of various poisons carried out by Bohorny (149) confirm the remarkable resistance of the myxoplasma 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 (150) 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, morpine and others which belong to the most dangerous poisons for higher animals may serve as growth substrate for yeasts and bacteria”.

Pfeffer (151) reports on the remarkable fact that some fungi take up arsenical 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 (150).

By contrast, according to Klebs (152) 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 (153). “The antlarax 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)
Myoplasma

- Hydrochloric acid: 1% — Applied for 48 hours, does not kill Bacillus anthracis (Dyrmont).
- Potassium hydroxide: 0.1% does not harm the typhoidal bacterium and 0.14% does not harm the cholera bacterium that live in gelatin (Kitsato)\(^1\).
- Copper sulfate: 0.1% disturbs the growth and assimilation of a yeast species;
  - At 1% mould is growing (Boekorny, p. 206), at 0.05% bacteria are growing.
- Zinc sulfate: "The life of rot fungi strangely isn't even hindered completely at 0.1% zinc sulphate" (Boekorny, l. c. S. 209).

Amoeboplasma

- 0.01% kills Paramaecium (Infusor) and zoospores.
- 0.1% currently kills all animals and plants.
- 0.01% kills infusoria: 1:50 000 kills all animals in 2 days (infusoria, rotifers, worms, insect larvae) and all plants (Cladospora, Convera, Spirogyra, Fauschieria): 1:100 000 000 slowly kills Spirogyra (l. c. S. 205).
- 0.01% kills infusoria in 24 hours (l. c. S. 205) and even 0.001% slowly kills them. Roots of planegam die at 0.02%.

[End Part III, vol. 30, No. 10, May 15, p. 347; Begin Part IV, vol. 31, 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, morphone, strychnine, serve as food for myxoids, 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"\(^2\). [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 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 myoplasma 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 menu consisting of protein, protoplasma, fat, starch and other carbohydrates. The myoplasma on the other hand, is all but impossible and is even satisfied by rough and undigestible food, a diet that would definitely kill every kind of amoeboplasma. — B e n e c k e \(^3\), for example, found a bacteria (Bacillus chitinovorus) feeding on chitin. The well known french bacteriologist M i q u e l \(^4\) observed bacteria that feed on rubber while assimilating a part of it and excreting H\(_2\)S. R a h n \(^5\) 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\(^6\) are members of this group. We have also seen that the myoplasma feeds on HCN, KCN, morphone, strychnine, and from chapter IV we have seen that the myoplasma, and only the myoplasma, 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 myoplasma 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 myoplasma is distinguished from the amoeboplasma by its resistance and robustness in general and by its greater ability to withstand harmful physical and chemical factors.

\(^1\) B e n e c k e , W., Über Bacillus chitinovorus, einen Chitin verzerzenden Spaltpilz. Botan. Ztg. 1905, Series I, p. 227.
\(^2\) P e r r i e r, L., Les colonies animales. 2. Edition, 1898, p. 39.
\(^3\) R a h n, O., Centralblatt für Bakteriologie (II), Vol. XVI, 1906, p. 382.
\(^4\) W e r d, H., Marshall, Onygena equina W ill i d, a horn destroying fungus. Philosoph. Transact. of the Royal Soc. London. Series B, Vol. 191, 1899, p. 269.
VIII. The other differences.

1. The mykoplasm is distinguished from the amoeboplasm by the presence of iron in a chemically fixed state. Reasons to postulate this comes from Macall on [14], and according to experiments of Raul in [16] and M o l i s c h [17], it appears as an essential constituent, also in fungi. It is generally known that without iron typical plasids cannot develop: lacking iron the plant becomes chlorotic, develops weakly and eventually withers. The chemical analysis of bacteria and fungi exhibits iron as well[18]: vinegar bacteria contain 8.15% FeO$_2$, lichen 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 fungus it increases up to 5% and this amount remains constant even in iron-poor soil.

[356] Should the observations of Justu s [19] be correct, that each nucleus contains iodine, it appears possible that the presence of this element may also reflect a specific character of the mykoplasm.

2. The mykoplasm of the free living mykoids is always surrounded by a cell wall, the amoeboplasm is often naked. But even in those cases where the amoeboplasm is surrounded by a cell wall as in plants, one encounters the deep differences between the cell walls of mykoids and amoebooids. Plants contain a cell wall made up of carbohydrates, mainly cellulose. This peculiarity led Bonnier and Leclerc du S a b l o n [20] 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 nitrogren-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 Sch m i d t and W e i s [21], similar to the protoplast, 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 [22] reports that the fungal cell wall consists of nitrogen and contains substances (chitin according to him), which are lacking in the Saprolegniaeae and Peronosporaeae, [357] i.e. in the phycomycetes[23], where the cell wall consists of cellulose, also confirmed microchemically by M a n g i e [24].

Finally, considering cyanobacteria, in which we may expect a cellulosic cell wall due to the presence of chlorophyll, K o h l [25] 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 mykoplasm and the amoeboplasm, one may still direct the attention towards certain morphological characters. Whoever compares the peculiar fruit bodies of cap muci nas, gastromycetes, or of the white rot fungi with a true plant, he is 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, of that of the plant and of that of the fungal kingdom, opens up a profound and principle difference[26].

Plants are made of true tis sue, 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.
4. We have good reasons to assume that the mycoplasma reveals a much more complex structure than the amoeboplasma. The reason is founded in the role that the mycoplasma plays with respect to inheritance. In case my theory regarding the origin of the cell nucleus is correct, which I would like to propose in the forthcoming article, the mycoplasma 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.  

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 myxoids 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.  

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.

Epoch I: Fiery glowing state of the Earth’s surface.  
Epoch II: The Earth is no longer glowing, but still very hot (more than 100 °C) and therefore absolutely dry.  
Epoch III: The surface of the Earth is covered with boiling or hot water with temperatures of 50–100 °C.  
Epoch IV: The water temperature falls below 50 °C. In which of these periods could life have emerged?

According to Pfüger’s 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 mycoplasma described above allow us to answer this question in more detail than was previously possible. [360] The mycoplasma 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 mycoplasma.

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]

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365 It is possible that the extraordinary complexity of the structure of mycoplasma 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 mycoplasma, which we can tentatively attribute to this plasma and which would explain its great resistance to high temperatures.

366 Pfüger, Uber die physiologische Verbreitung in den lebendigen Organismen. — Pfüger, Arch. f. Physiol., Vol. X, 1875.
### Requirements
which necessarily have to be met by the first organisms.

1. Minimal size, inaccessible to the microscope.
2. Absence of organization.
3. Ability to withstand high temperatures close to the boiling point.
4. Ability to live without oxygen.
5. Ability 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.

### Attributes
of the bacteria that match the requirements.

1. The bacterial fogs consist of bacteria like organisms that are invisible under the microscope — the biococci.\(^{397}\)
2. At such a small size, biococci cannot have organization, following the law of dependence of organization upon size.
3. Bacteria tolerate temperatures up to 98 °C in the vegetative state and up to 150 °C in the reproductive state.
4. The vast majority of bacteria can live without oxygen.
5. The bacteria are able to synthesize proteins and carbohydrates (the latter without the help of chlorophyll) from inorganic substances.
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 Pfüger's hypothesis for the origin of life on Earth, which Verrawm says there is not a single fact contradictory to it.

Placing the origin of the mycoplasma within epoch III of the Earth's history, which follows as a consequence of the theory of the two plasma formations, fits in well with Pfüger'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 protoplasts from cyanistic 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 so 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 mycoplasma 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 [Baustein] 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 mycoplasma dissipated and the further evolution of life was only possible following the principle: omne vivum e vivó. In this way the most prominent distinguishing character of new organisms, 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 mycoplasma thus emerges from growth of the original mycoplasma, as its direct continuation [als deren 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 hospitable compared to those at the formation of the mycoplasma. They elicited the very different properties that characterize the amoeboplasma.

[363] This type of plasma probably arose in the form of small clumps, as small ascellular Monera that crawled like amoebeae 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 micococci, living unored at first and dispersed within the Monera cell body, then in the form of a distinct group assembled in the cell's centre and finally surrounding themselves by a membrane [Häußen], 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 anacell 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 amaccle 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

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\(^{397}\) See above: Lüffler and Froesch, Berichte der Kommission zur Erforschung der Maul- und Klauenseuche bei dem Institut für Infektionskrankheiten in Berlin, Centralbl. f. Bakter., Series 1, Vol. XXII, p. 371. — No cardet Roux, Annales del Instituto Pasteur, 1899, No. 4. — Eters, L, Recueil de l'Institut botanique, Université de Bruxelles, 1903. — Lasar, Handb. d. techn. Mykol., Vol. 1, 1904, p. 32 and 35.

\(^{199}\) 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.
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 organism, 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 symbioses²⁹⁰. 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. Plantids
  - 2. Chromatin granules of nuclei
    - a) Algae (autotrophic organisms)
    - b) Leucohyphaceae (heterotrophic organisms, Phycocyanes)

### II. The plant kingdom
(two-fold symbiosis)
- 1. Algophyta
- 2. Lyyophyta
- 3. Phylaephyta
- 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. 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 cellulose²⁹¹ | 4. The cell walls consist of fungus 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, occasionally with periplasm. | 5. Spores always have a membrane, they are formed by internal depositions of individual parts from the general mass of plasma, epiplasm always present. |

²⁹⁰ The lichens represent a threefold symbiosis.
²⁹¹ 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. M. V. van Wisselingh did not.
Also, morphologically the phycocyanetes 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. Therefore I recognize the phycocyanetes as a side branch (more exactly as several side branches) of the algae and find it necessary to replace the inappropriate term Phycocyanetes with the new term — Leucophyceae. 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.

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325 From this point of view it would be extremely interesting to study a number of fungi which are usually classified as ascomycetes: Ascocida, 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 amoeboid movement, similar to that of the Leucophyceae, whether epiplasma 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.

326 Some authors are already inclined to this point of view, although the majority of botanists (Brefeld, Blakman, Harper [1900], Barker [1903], H. Fischer [1904], Dangeard [1896–1905]) continue to derive the fungi from the phycocyanetes.
In reality there are no such transitional organisms because there is no transition between symbiosis and nonsymbiosis. 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—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 myxoid.

All of the foregoing is summarized in the accompanying figure.

In the figure, the myxoplasma 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, which descend from two independent roots. The phylum on the left is composed of the ur bacteria—bilococci, it is the kingdom of the myxoids 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 phylum, 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), forming 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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36 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 in nature.
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