Resumen por el autor, Ivan E. Wallin.

Sobre la naturaleza de las mitocondrias.

III. La demonstración de las mitocondrias mediante los métodos
bacteriológicos.

El autor ha preparado frotes de tejidos animales en porta-
objetos. Las mitocondrias se tiñen con los colorantes bacterio-
lógicos en estas preparaciones.

IV. Un estudio comparativo de la morfogénesis de las bacterias
de los nódulos de las raíces y de los cloroplastos.

El Bacillus radicicola experimenta un desarrollo morfológico
en los nódulos de las raíces, semejante a la morfogénesis de los
cloroplastos. Una nueva forma de bacteria ha sido descubierta
por el autor, quien discute la simbiosis y hace notar la analogía
de la simbiosis en los líquenes con su concepto de las mitocondrias.

ON THE NATURE OF MITOCHONDRIA

III. THE DEMONSTRATION OF MITOCHONDRIA BY BACTERIOLOGICAL METHODS

IV. A COMPARATIVE STUDY OF THE MORPHOGENESIS OF ROOT-NODULE BACTERIA AND CHLOROPLASTS

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TWO PLATES (NINE FIGURES)

III. THE DEMONSTRATION OF MITOCHONDRIA BY BACTERIOLOGICAL METHODS

In a former paper ('22) the author submitted evidence to show that the special mitochondrial technique in general use, including the vital janus-green method, is not specific for mitochondria, but will also stain bacteria. While, from a purely theoretical consideration, the properties of mitochondria are of such a nature that one could hardly expect them to respond to bacteriological methods, an analysis of results in this direction is not only interesting, but also instructive.

The author has not been able to find any references in the literature to mitochondrial staining with bacteriological methods. The results recorded below will serve to indicate not only the reactions of mitochondria to such methods, but will also indicate possibilities in mitochondrial manipulation.

MATERIALS AND METHODS

The tissues used in this study have consisted of various samples from young rabbits, kittens, and mature dogs. These tissues have included lymph nodes, liver, pancreas, kidney, salivary glands, suparenal, thymus, and other tissues. Immediately after

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removal from the previously killed animal, smears of the organs and tissues were made on microscopical slides. The smears were then permitted to dry in the air without any other fixation.

A large number of bacterial staining methods were later applied to the smear preparations. Most of these staining methods have no selective action on bacteria, and consequently the entire smear was stained and mitochondria could not be distinguished with any important degree of clarity. In a few instances Gram's stain appeared to give a little clearer differentiation. One bacterial staining method was found, however, that gave a sharp differentiation—Pappenheim's pyronin-methyl green. This stain has had very extensive use in bacteriological technique. Todd ('18) recommends it especially for the demonstration of bacteria in cells on account of its selective action. In this study saturated aqueous solutions of pyronin and methyl green have been used in various proportions of mixture. In some instances it was found that a special proportion of the two stains was necessary to produce sharp differentiation. As Todd has recommended for the demonstration of bacteria, it is necessary to experiment with the proportions of the two stains to attain the best results.

RESULTS OF BACTERIOLOGICAL STAINING METHODS ON TISSUE SMEARS

Figure 6 is a camera-lucida drawing of a part of a young rabbit pancreas smear after pyronin-methyl-green staining. There was only a small area in the entire smear that appeared like the illustration. It is only in a very few cases out of a great number of attempts that anything resembling mitochondria were present in pancreas preparations. The bodies in the smear, represented in figure 6, appear like mitochondria, but not like the typical mitochondria of pancreas cells. Obviously, I am not in a position to definitely state that these bodies are mitochondria. It appears probable to the author that these bodies are the fragments of the original mitochondria of the pancreas cells. They may be artifacts. If they are, then, what evidence do we have of the reality of mitochondria in stained preparations?

Figure 7 is a camera-lucida drawing of a part of a rabbit-liver smear after pyronin-methyl-green staining. The small stained bodies in this preparation appear like the typical mitochondria of liver cells. I can find no alibi for their mitochondrial nature.

Figure 8 is a camera-lucida drawing of a portion of a rabbit-kidney smear after pyronin-methyl-green staining. It is difficult to find a place in the kidney smears where mitochondria-like bodies are distinct or present. The group of kidney cells illustrated was more or less intact. The minute bodies in the illustration were sharply differentiated and are not unlike the typical kidney mitochondria.

Figure 9 is a camera-lucida drawing of a part of a rabbit lymph-node smear after pyronin-methyl-green staining. The minute bodies represented in the illustration were sharply differentiated not only in the cells intact, but also in the cytoplasm of ruptured cells. They appear like the typical lymph-node mitochondria.

In a number of lymph-node-smear preparations it was observed that in some cells the entire cytoplasm, which was apparently intact, was homogenously stained with pyronin (the member of this stain combination which stains the mitochondria). Further, in practically all tissue-smear preparations, the ruptured cytoplasm is distinctly stained by the pyronin. This latter action of the stain is interesting on account of the supposition (original contention of Pappenheim?) that pyronin-methyl green is a selective stain for certain lymphocytes, staining their cytoplasm pink or red.

It appears reasonable to the author that in the cases where the cytoplasm stains with the pyronin the mitochondrial substance has diffused into the cytoplasm. If this interpretation is correct, it assumes that mitochondria are composed of a substance that is miscible with cytoplasm. It, further, assumes that under normal conditions mitochondria have a wall, pellicle, outer membrane, or some limiting structure. The diffuse character of the mitochondrial substance has also been observed in the ordinary fixed and stained mitochondrial preparations of lymph nodes.

These illustrations, it seems to me, are sufficient evidence that mitochondria may be demonstrated by bacteriological methods. In this work various problems in connection with the behavior of mitochondria have arisen. These problems have no fundamental bearing on the major problem of these studies, and consequently have not been pursued.

DISCUSSION

Aside from the demonstration of staining mitochondria by means of bacterial technique, certain facts observed in this study are of importance in connection with the main problem.

In a number of attempts to demonstrate the mitochondria in adult dog tissues with the above-described bacteriological technique, I have practically failed. Only in a very few instances have I been able to distinguish mitochondria-like structure in these preparations. These attempts were made with stains from a different source than the ones originally used on kitten and rabbit tissues. The original were Grübler's stains. However, with the kitten and rabbit tissues the results were not the same for all tissues. It was exceedingly difficult to demonstrate any mitochondria-like bodies in the pancreas. I have not been able to demonstrate any mitochondria in the salivary glands, thyreoid, and suprarenal. On the other hand, it appears quite easy to stain the mitochondria in the cells of lymph nodes. It must be borne in mind that in all these preparations the tissues were crushed when the smear was made.

Two prominent facts stand out in these results: first, mitochondria are not as delicate as it has been supposed; second, mitochondria vary in fragility.

Regarding the delicacy of mitochondria, Cowdry ('18) maintains that the slightest desiccation of a tissue is sufficient to alter them. The above-recorded results with bacteriological methods demonstrate the fact that mitochondria may retain their form and be stained after the degree of desiccation present from complete drying in the air.

These staining reactions, further, indicate the danger in formulating hypothesis and drawing too extensive conclusions on the basis of staining reaction alone.

IV. A COMPARATIVE STUDY OF THE MORPHOGENESIS OF ROOT-NODULE BACTERIA AND CHLOROPLASTS

In a growing conception of a bacterial nature of mitochondria, one naturally would seek an example of an undisputed symbiotic bacterium for study. The root-nodule bacteria, Bacillus radicicola offers an example of a relationship between two organisms that is, at least, of a partial symbiotic nature. The bacterial organism in this case, apparently, exists as a free-living organism in the soil. Under favorable circumstances, it enters the root hairs of Leguminosae and ultimately may be found in the cytoplasm of the cells of the root-nodules. The host plant responds to the infection by developing the root-nodules.

The degree of symbiosis in this example is, perhaps, not absolute.[1] The bacterium can live as an independent organism in the soil. Its symbiotic qualities are of the nature of partial adjustment. In other words, the organism has not changed to such a degree that it cannot exist independently of the host organism. This status of its existence is undoubtedly responsible for the ease with which the organisms from a root-nodule may be grown on artificial culture media.

Compared to absolute parasitism, the root-nodule bacteria are not as dependent on the host as is an absolute parasite. In the case of an absolute parasite, as well as an absolute symbiont,[2] the adjustment is so complete that the organism does not normally live outside the host. However, the Bacillus radicicola offers an illustration of a microscopic organism that may live and flourish within the cytoplasm of the cells of a higher organism.

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[1] Various classifications of symbiosis may be found in the literature. The terms employed in these classifications have been based upon individual examples and conceptions and consequently can not be employed with clarity in an enlarged conception of symbiosis. Schneider's (1897) terms "mutualism," "individualism," and "contingent mutualism" are vague and misleading. The terms 'absolute' and 'incomplete' symbiosis have been introduced by the author on account of their simplicity, clearness, and direct significance.

[2] The author chooses to use the term "symbiont" employed by Schneider (1897) in preference to the term "symbiote" introduced by Portier (1918) for the reason that "symbiont" refers to either one of the two organisms entering into symbiosis, while "symbiote" refers particularly to mitochondria in a bacterial conception of their nature.

Further, it offers evidence of the fact that the chemical products of the bacterium in this case are essential to the life of the host plant. True, the host plant may procure these chemicals by absorption from the soil, and it may even do so in spite of the nodule organisms. This fact, per se, has no bearing on the importance of the phenomenon. The point at issue may be clarified by an illustration: The thyreoid gland produces a chemical substance that is essential to normal metabolism in higher animals. The gland may be removed from an animal, but the chemical substance must be supplied artificially if life is to be maintained normally. If it were possible to stimulate the production of this chemical substance from another organ of the animal, then the thyreoid would be unessential and in all probability would degenerate.

Lewitsky ('10), Guilliermond ('12), Regaud ('11), and other investigators have ascribed to mitochondria the property of plastid formation in plants. According to these investigators, the original mitochondria transform into plastids. Accompanying this transformation the mitochondria take on the various functions characteristic of plastids. Various kinds of plastids are to be found in plants. From the standpoint of evolution, the more important of these plastids are the chloroplasts, or the plastids containing chlorophyl.

According to Guilliermond, chloroplasts in higher plants are formed from mitochondria. He has, apparently, observed the various intermediate stages in the metamorphosis from a minute body, the mitochondrium, to a fully formed chloroplast. Such a morphogenesis is so strikingly similar to the morphogenesis of the Bacillus radicicola in the root-nodules of Leguminosae that the writer feels justified in presenting his observations on these forms.

MATERIALS AND METHODS

Root-nodules found on the roots of the common white clover, growing on the University campus, were fixed in the modified Flemming's fixative described in a former paper (Wallin, '22). After they were washed, dehydrated, cleared, and embedded

in paraffin, they were cut into sections 3μ in thickness, mounted, and stained with Bensley's aniline fuchsin methyl green.

The author has made no original observations on chloroplast formation. The work of Lewitsky, Regaud, Guilliermond, and other investigators in this field is accepted as fully demonstrated.

Bacillus radicicola

A longitudinal section of a root-nodule of the white clover, when examined with a low magnification of the microscope, reveals three distinct areas in the nodule. When a higher magnification is employed, the three distinct areas may be seen to be composed of cells containing three distinct types of bacteria-like organisms. Figures 1, 2, and 3 are camera-lucida drawings of portions of these three areas from a single nodule.

In figure 1 the typical Bacillus radicicola may be recognized. Most of the organisms are rod-shaped. A few of the characteristic Y-shaped individuals may be seen. It is this type, represented in figure 1, that, I believe, is generally considered the active individuals in nitrogen fixation. They may be designated the 'mature forms.'

In figure 3 the cells contain small bodies that are not unlike mitochondria in appearance. These forms, undoubtedly, are the young bacilli that have recently entered the nodule, or they represent a young form that will later metamorphose into the mature type. They may be designated the 'juvenile forms.'

Prazmowski (quoted by Marshall, '12) and other investigators have demonstrated that the source of Bacillus radicicola is from the soil. The free-living forms are quite small. They enter the roots through the root-hairs. The host plant responds with the production of the nodules into which the juvenile forms migrate and later metamorphose into the mature type. I have not been able to find a comprehensive description of the metamorphosis.

In figure 2 the organisms found in the cytoplasm of the nodule cells are spherical in shape. I have been unable to find any mention of these forms in bacteriological literature. The American text-books on bacteriology have no reference to them. They may be designated the 'senile forms.'

These senile forms occupy the older part of the nodule. That is, they are present almost exclusively in the cells of the nodule nearest to the attachment to the root. Apparently, there is nothing indicated concerning their activities. Their position in the nodule suggests the possibility that they are senile. It is possible that they no longer concern themselves with nitrogen fixation. However, one is justified in concluding that they are a stage in the morphogenesis of Bacillus radicicola.

The failure of investigators of this interesting and well-known organism to notice the existence of the senile forms is, assuredly, an excusable error. The object of my study, as well as my histological training, suggested the study of the root-nodule cells intact. The bacteriologist would crush the nodule and make a smear to study the contained bacteria. Again, the bacteriologist is interested in the cultural behavior of bacteria. The nodule must be crushed to transplant the contained organisms.

I have never observed the spherical or senile forms of the bacillus in smear preparations of the root-nodules.

Figure 4 is a camera-lucida drawing of a portion of the stem of a clover leaf after mitochondrial fixation and staining. Figure 5 is a camera-lucida drawing of a portion of an unfolded clover leaf after mitochondrial fixation and staining. These preparations are introduced to furnish a basis for comparison of apparent mitochondria in this plant with, particularly, the juvenile forms of bacillus radicicola as represented in figure 3. It is apparent from an examination of these illustrations that the mitochondria are similar in appearance to the juvenile bacteria. While it is obvious that form in itself does not necessarily indicate relationship, the question may properly be asked: What evidence is there to indicate that these structures are cytoplasmic organs and not representatives of the organisms that are known to be present in the root-nodules of this plant? Again, it may be asked with equal pertinence: What evidence may be submitted to indicate that these bodies, generally considered cytoplasmic organs, are not bacteria that have gained entrance to the plant through the root-hairs? The author has not been able to find any evidence that would satisfactorily answer these questions.

To recapitulate, the Bacillus radicicola is a minute organism that may be found as a free-living bacterium in the soil. Under favorable conditions, it may enter the root-hairs of Leguminosae and enter into a partial symbiotic existence. The host plant responds to the infection with a production of nodule cells. The invading organism comes to lodge in the cytoplasm of the root-nodule cells. In a mature root-nodule these forms, apparently, represent three stages in the morphogenesis of the bacillus after it acquires the symbiotic relationship.

DISCUSSION

The morphogenesis of Bacillus radicicola from the juvenile to the senile forms is strikingly suggestive of the morphogenesis of chloroplasts as described by Guilliermond and other investigators. Both forms develop from minute structures that have similar form and staining reactions. Both forms have a similar shape when they have reached their fullest development and are apparently alike in fragility. Both forms have a similar relationship to the cytoplasm of host cells. The morphogenesis of chloroplasts, as described by Guilliermond, appears to imitate more closely the morphogenesis of an organism than the development of a cytoplasmic organ.

The senile forms of Bacillus radicicola have a particular interest and will serve a useful existence in our conception of the nature of mitochondria. It is quite obvious that absence of these forms in bacteriological literature is due to the fragility of the organism. In ordinary bacteriological technique these forms are destroyed; both in smear preparations and planting on culture media they would be destroyed in the procedure. I have not used any other fixation for root-nodules than the one indicated above (a mitochondrial fixative). It is probable that the senile forms are destroyed by many fixatives just as mitochondria are, and this condition may account for some of the failures of previous investigators to observe them.

If my interpretation of the senile forms is correct, then, they furnish excellent proof of the contention I stated in the 'ad-

dendum' to a former article (Wallin, '22), namely, that fragility is a resultant of symbiosis. Further, their fragility refutes Regaud's contention that no bacteria are known which exhibit the fragility of mitochondria.

The apparent fragility of the senile forms of Bacillus radicicola has a bearing on the problem of growing mitochondria on artificial culture media. It is apparent from the absence of these forms in bacteriological literature that they have not been cultivated. This failure to grow them on culture media may be caused by various factors. It may be possible that they require a special culture medium. It is more likely, however, that their fragility does not permit a transfer. A third possibility presents itself, namely, that they may have lost the power of reproduction. This latter possibility appears the least likely explanation. It it quite certain that if success is to attend attempts to grow mitochondria in artificial culture media, the fragility of mitochondria, as well as the proper culture medium, must be taken into account.

There is no reason to suppose that all mitochondria and symbiotic bacteria develop and possess the same degree of fragility. To the contrary, I have presented evidence in the preceding section of this paper in support of the contention that mitochondria vary in fragility.

It is perhaps excusable at this time to digress into the realm of theory. The question naturally arises: Would a symbiotic bacterial interpretation of mitochondria be antagonistic to established factors in the theory of evolution?

Osborne ('17) has stated a clear and logical conception of evolution on the earth. His conception includes a chemical evolution preceding and leading up to the establishment of definite organisms. The first organisms in this conception were of a bacterial nature. These primordial bacteria or bacteria-like organisms were able to subsist on inorganic material. This conception of the first life is strengthened by the discoveries of Alfred Fischer ('00) and other later investigators. Fischer discovered a strain of bacteria that apparently represent the persistence of these primordial organisms, the Nitroso monas.

The presence of these primordial organisms in the beginning of evolution established the conditions essential for the development of those higher forms that require organic material for sustenance. It is not difficult to conceive the rôle that the primordial chlorophyl-bearing organisms played in the modus operandi of early evolution. Concerning themselves with the production of starch, the most important of the early food materials, they undoubtedly assumed the rôle of food factories preparing the way for the evolution of higher life.

The simplest organisms containing chlorophyl are bacteria or bacteria-like organisms. These organisms are generally called the blue-green algae. Campbell ('99), Thom ('12), Osborne ('17), and other investigators, however, maintain that they are more closely related to bacteria than to algae. Regardless of their relationship, whether with the bacteria or with the algae, the fact remains that chlorophyl is one of the oldest specialized materials of living matter.

It is in harmony with known biological behavior to conceive of chloroplasts not as organs developed in the cytoplasm of higher plants, but to look upon them as bacteria or bacteria-like organisms that accepted the leisure of a symbiotic partnership in the struggle for existence. In exchange for the nourishment supplied to it by the host plant, the invading symbiont furnishes an indispensable product to the host organism. It is obvious that if this interpretation coincides with reality, then the chloroplast is an example of *absolute symbiosis*.

The presence of chloroplasts in certain animal cells makes the foregoing hypothesis all the more alluring. It is true, that in some of these animal cells the chlorophyl-bearing bodies are unicellular algae that have been ingested by the host organism. This fact, however, serves to demonstrate the possibility that certain animal cells are so chemically constituted that algae may live within their cytoplasm with apparent impunity. The possibility also suggests itself that unicellular chlorophyl-bearing organisms may develop a symbiotic relationship with some animals and that the products of the chlorophyl-bearing organisms may be essential to the sustenance of the animal cell.

In a discussion of algae and their relationships, Campbell
('99) refers to some chlorophyl-bearing algae that have developed
a symbiotic relationship to some higher plants. There is no
reason to suppose that all unicellular green or blue-green algae
belong to a single species. To the contrary, botanists recognize
many species. The fact that some species of algae may be
recognized in a symbiotic relationship with higher plants indicates
the possibility that other species may have developed an absolute
symbiosis with some higher plants. After an organism has de-
veloped absolute symbiosis, it is not conceivable that it is capable
of an independent existence under natural conditions. It is,
also, to be expected that an absolute symbiont would lose some
characters and properties that the free-living progenitor possessed.

One of the best known and oldest examples of symbiosis is
furnished by the lichens. This symbiosis was first described by
Schwendenen in 1860, and has since been confirmed by the in-
vestigations of a host of authors. The lichens furnish not only
an indisputable example of absolute symbiosis, but also varying
degrees of symbiotic relationships.

In the lower lichens the algal symbiont is capable of independ-
ent existence. While it has not been demonstrated, Schneider
('97) and other investigators believe that the fungal symbiont
in some lowly lichen may also be capable of independent exist-
ence. In the most highly developed lichens the symbiosis is
complete or absolute; neither symbiont can live without the
other. Here we have an example of symbiosis that, apparently,
is identical with every detail in the relationship of chloroplast to
host plant.

Between the most lowly and the most highly developed lichens,
various degrees of symbiosis are represented. This gradation of
symbiosis is accompanied by differences in reproduction. In the
lowly lichens, where symbiosis is incomplete, the algal and fungal
symbionts reproduce independently. In higher forms, but where
the symbiosis is still incomplete, an accessory reproduction may
take place by a type of budding in which both algal and fungal
symbionts are represented. In the most highly developed lichens,
where there is absolute symbiosis, both the algal and fungal

symbionts enter into the formation of the reproductive organ. This organ which is comparable to the germ cells of higher organisms was first named 'soredium' by Acharius (from Fünfstück, '07). Various types of soredia may be found in different lichens. In general, the fungal symbiont supplies a part (very much of the character of the spore organ in fungi) which arranges itself around the algal contribution (the gonidia). In other words, *the algal symbiont is carried from one generation to another in the reproductive element of the fungal symbiont.* This condition is absolute symbiosis in its highest development.

This latter reproductive phenomenon indicates the solution to a problem that will assuredly arise in connection with a symbiotic bacterial conception of mitochondria: If mitochondria are symbiotic bacteria, what is their source? Indeed, we would not seek some avenue of entrance in the adult organism nor in the embryonic body. The search would logically begin in the germ cell. The presence of mitochondria in the germ cell has been fully demonstrated. To seek the original source of a symbiotic-bacterial mitochondrium would lead us back to the dawn of evolution. Such an attempt, per se, is impossible. On the basis of biological behavior as exemplified in the absolute symbiotic lichens, there is but one logical answer to the above-stated question: *The symbiotic-bacterial-mitochondria are carried from one generation to another in the germ cells.*

Another question presents itself in connection with a symbiotic bacterial theory of mitochondria: Does such a theory harmonize with the known factors of cell activity? Altmann ('90) originally advanced the theory that the 'bioblasts' (mitochondria) are the ultimate units of life and the cytoplasm of the cells in which they are contained is lifeless. Altmann's ideas, apparently, were chiefly theoretical. Verworn ('99) and other investigators demonstrated the untenable position of such a hypothesis. Abundant evidence may be offered to prove that cytoplasm, by itself, possesses properties that are characteristic of living matter. If Altmann had limited his dissertation to a consideration of the 'bioblasts' alone, the burden of proof would have fallen on his adversaries. He misled his critics by introducing an erroneous conception of cytoplasm. This brought forth ridicule from his

critics instead of further scientific analysis. Following Altmann's dissertation, a great amount of theory has been advanced concerning mitochondria and cytoplasm. If we analyze many of these theories we find very little if any real evidence as a basis for their pronouncement.

The cell may be considered a unit chemical factory into which materials are almost continually passing and products are being eliminated. The simplest type of cell, if it may be considered a cell, is the bacterial organism. It is simple in the sense that it responds more readily to environmental conditions than do the highly differentiated cells of complex organisms. Gourney-Dixon ('20) has collected a mass of evidence in this direction. During recent years there has been a great deal of evidence submitted to show that the cells of one tissue are dependent upon the product of the cells of other tissues. Adami ('10) even goes so far as to state that every cell in a complex organism performs the function of internal secretion. Such a relationship of cells is also present among the lowly bacteria and is designated as symbiosis by bacteriologists. This principle of cell dependence is, thus, not characteristic of the cells of higher organisms, but is a primitive adjustment. Further, it is highly probable that this principle of cell dependence has been a fundamental factor in organic evolution.

The symbiotic bacterial conception of mitochondria, apparently, is not antagonistic to any known principles of cell activity. On the other hand, such a conception only extends the 'cell dependence principle' to include an intimate dependence of all highly organized cells on the activities of simple cells. The Bacillus radicicola in the root-nodules of Leguminosae is incontrovertible testimony that such a functional and morphological relationship does exist.

The dependence of higher life on bacteria in a different sense has been embodied into the biological conceptions of all students in biology. The principle of the 'nitrogen cycle' stands sponsor to these conceptions. It is within the domain of logic to extend this well-established principle of dependence of higher life on bacteria to include the more intimate dependence of life processes on bacteria.

GENERAL RÉSUMÉ

From the evidence that has been submitted in this study, including sections I and II in a former paper, the author believes that he has demonstrated the following facts:

There is no fundamental difference in the staining reactions of mitochondria and bacteria.

There is no fundamental difference in the reactions of bacteria and mitochondria to certain chemicals that have been used in attempting to determine the chemical nature of mitochondria.

Mitochondria may be demonstrated by bacteriological methods.

Bacteria may be demonstrated by mitochondrial methods.

Mitochondria vary in fragility.

Bacteria vary in fragility.

Mitochondrial substance is apparently miscible with the cytoplasm of the host cell.

The author, further, believes that the following apparent facts and biological principles support a bacterial nature of mitochondria:

Similarity of form.

Similarity in staining reaction.

Similarity in chemical ractions.

Similarity in physical properties (fragility).

Similarity in functional properties (synthesis).

In harmony with principle of biological behavior as exemplified in the 'struggle for existence' resulting in symbiosis.

In harmony with known factors of evolution.

In harmony with known factors of cell activity.

In harmony with 'principle of cell dependence.'

In harmony with the principle of analogy (Bacillus radicicola, lichens).

CONCLUSIONS

In a 'balance sheet' of favorable and unfavorable evidence in behalf of a bacterial nature of mitochondria, it appears to the author that the 'unfavorable' side of the 'sheet' lacks entries. After careful analysis, the author is convinced that no property of mitochondria has been recorded in the literature that is not equally applicable to bacteria.

From the evidence that has been recorded in these studies, together with the evidence that may be found in mitochondrial literature, the author can arrive at no other conclusion than, that *mitochondria are symbiotic bacteria in the cytoplasm of the cells of all higher organisms whose symbiotic existence had its inception at the dawn of phylogenetic evolution.* The conception embodied in this conclusion presupposes that the establishment of new symbiotic complexes is coexistent with the development of new species.

These studies have been pursued by the author independently. The conceptions and principles stated in this article have been formulated entirely on the basis of evidence that the author has gathered. Portier ('18) has arrived at a similar conception of the nature of mitochondria. His treatise 'Les Symbiotes' has not as yet been read by the author. A critical analysis of Portier's book will be undertaken by the author in the near future.[3]

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[3] After this article had been received by the publishers I found a reference to the spherical forms of Bacillus radicicola. Löhnis (1921 Studies upon the life cycles of bacteria. Pt. 1. Memoirs of the National Academy of Sciences, Vol. XVI, 2nd memoir. Wash.) has described spherical forms of this organism. The spherical forms are of different sizes and, according to Löhnis, represent morphogenic stages in the life cycle of Bacillus radicicola.

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EXPLANATION OF PLATES

The figures were made with the aid of the camera lucida. The lenses used were: 2-mm. apoch. oil.-immer. obj., comp. ocular no. 8. The figures in plate 1 are reduced one-half from the original. The figures in plate 2 are reduced only very little from the original.

PLATE 1

EXPLANATION OF FIGURES

1   Mature forms of Bacillus radicicola in the cytoplasm of root-nodule cells. Bensley's stain.

2   Senile forms of Bacillus radicicola in the cytoplasm of root-nodule cells. Bensley's stain.

3   Juvenile forms of Bacillus radicicola in the cytoplasm of root-nodule cells. Bensley's stain.

4   Mitochondria in the leaf stem of the white clover.   Bensley's stain.

5   Mitochondria in a young unfolded leaf of the white clover.   Bensley's stain.

6   Rabbit pancreas smear after pyronin-methyl green staining.

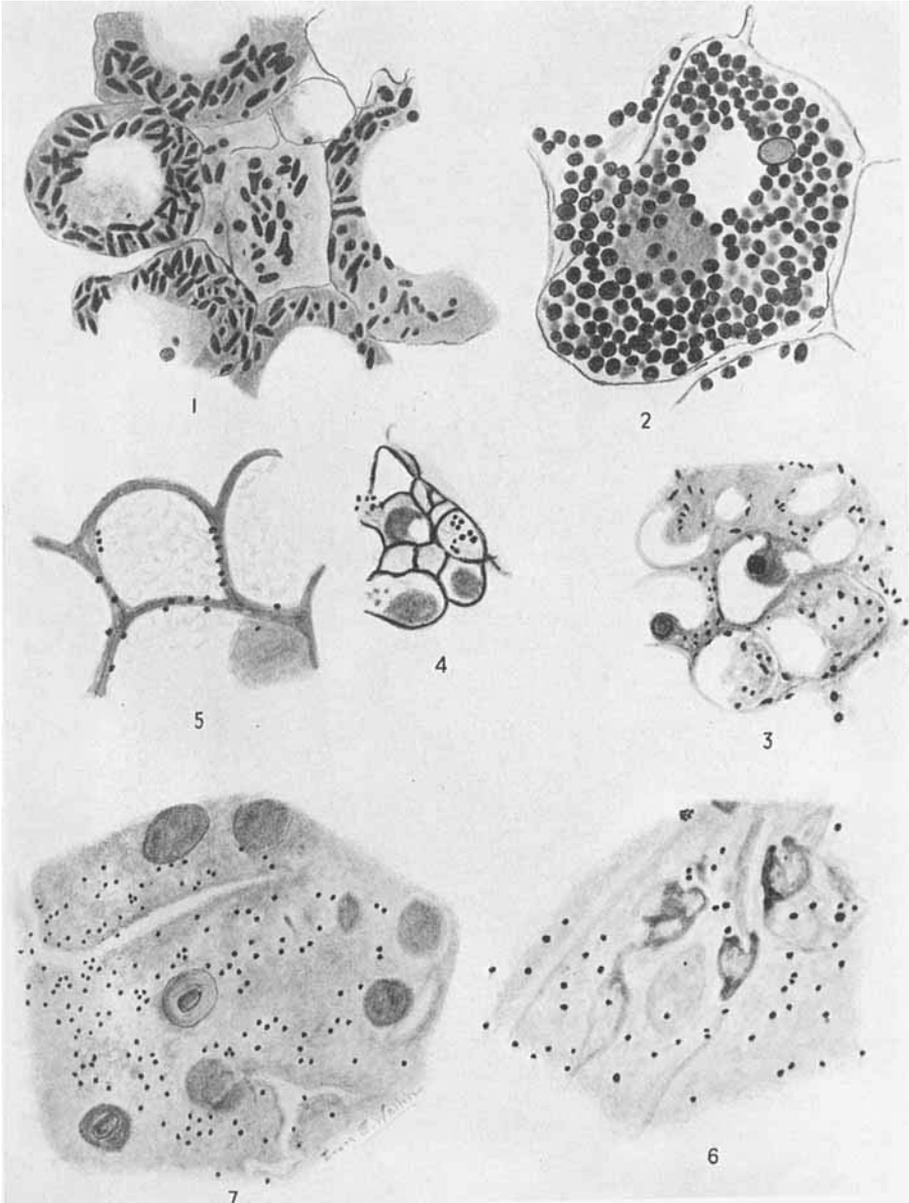7   Rabbit liver smear after pyronin-methyl-green staining.

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PLATE 2

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