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Wissenschaftliche Original-Mittheilungen.*)

The Relations of Chloroplastid and Cytoplasma.

By

Alfred J. Ewart, D. Sc., Ph. D.

Deputy Professor of Botany in Mason College. Birmingham.

In a recent paper Kny and Kolkwitz have published a series of observations, carried out by means of the *Bacterium* method, bearing on this subject. On some points Kny and Kolkwitz obtain apparently contradictory results to those given in the paper read by me two years ago before the Linnean Society. (A[1]). The authors, unfortunately, seem only to be acquainted with the abstract of this paper published by Pfeffer in the Ber. der K. S. wiss Ges. in 1896. Surely to criticize any positive results

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without having seen the original paper giving an account of them is rather a hazardous proceeding.

On the question as to whether the isolated chlorophyll body is capable of independent CO_2 — assimilation Kny and Kolkwitz obtain contradictory results to Engelmann, Haberlandt and myself. The explanation of their failure to obtain positive results is seen at once on referring to the text. In the first place the authors worked in nearly all cases with impure cultures, made, without adopting any precautions whatever, by allowing meat to decompose in water. The resulting *Bacterium* containing putrescent and poisonous fluid was markedly alkaline, but was, nevertheless, added directly to the isolated chlorophyll grains. It has however been shown (p. 415 A[1]) that even weak alkaline solutions exercise a marked injurious effect upon the chlorophyll grain, even when in the intact cell, depressing or inhibiting its assimilatory powers. Naturally the isolated chlorophyll body will be very much more sensitive.

To obtain isolated chlorophyll grains Kny and Kolkwitz adopted a different method to that employed by Haberlandt and myself. Their method does not seem, to judge by their negative results, as capable of yielding uninjured chlorophyll grains, as the simpler method, in which everything depends upon the manipulative skill of the operator.

A third and most fatal objection lies in the statement by the authors, that they found ringing the coverslip with vaseline to be, in the absence of air bubbles and assimilating organisms, unnecessary! As has been already shown, the most careful ringing to exclude all external oxygen is an absolute necessity for accurate experimentation. Thus in thinly ringed cell preparations, even if a few Bacteria are enclosed, sufficient oxygen may diffuse in to keep an end cell of Chara alive and shewing slow rotation for a period of days extending to more than a week in some cases, though kept in continuous darkness. (p. 420 A[1]).

I have repeated some of my previous observations, using unringed cell preparations but have found it quite impossible under such conditions to obtain any reliable results. The reason for this is twofold. Firstly, the most actively reacting *Bacteria*, collect at the edges of the coverslip where there is an abundance of oxygen and leave the centre of the field where there is but very little oxygen; and secondly, owing to the evolution of oxygen from the isolated chloroplastid being always weaker, and generally much weaker, than from an algal cell of the same size or from the same normal grain, the amount of oxygen which it evolves is insufficient owing to the relative abundance and hence comparatively high partial pressure of the surrounding dissolved oxygen which has diffused in at the open edge of the coverslip, to markedly attract the surrounding bacteria in the centre of the preparation, which, it is worthy of notice, are, as has been seen above, the less actively re-acting ones.

Kny states, that, in certain cases, chloroplastids, with a little plasma attached, may continue to evolve oxygen for a time and apparently assimilate. My own observation was, that such were less likely to show any evolution of oxygen, than ones which were quite isolated (p. 426 A[1]) It is possible that what Kny observed was a chemotaxic attraction of the *Bacteria* to the fragment of dying plasma by the nutritious juices exuding from it, this and the presence of a certain amount of oxygen in the unringed preparations permitting the movement of the Bacteria to continue for a time in the neighbourhood of such fragments of plasma. (See. pp. 366, 367, 418 A[1]).

A fourth source of error arises from the fact that the illumination employed by Kny was so intense that when any examinations were made it must be weakened to avoid injury to the eye. I have found that any concentration of the light, if ordinary diffused daylight is used, beyond that afforded by a mirror and Abbé condenser, soon reacts unfavourably upon the chlorophyll grain whether isolated or intact in the cell, causing finally a diminution of their assimilatory powers, and a fading of the chlorophyll. (See. p. 439 A[1] and p. 439—446 A[4])

At the some time concentrated illumination, diminishes the sensitivity and the rapidity of movement of the *Bacteria* and causes them finally to come permanently to rest. Both these results, are, as Pringsheim and others have shown, rapidly produced if concentrated sunlight is employed, but, in the tropics, even using the light directly reflected from a cloud obliquely illuminated by the sun, with no further concentration than by an ordinary microscope mirror and an Abbé condenser, a similar, though much weaker, effect may be produced, if only the period of exposure be sufficiently long. It is extremely probable that there are optimal and maximal as well as minimal intensities of illumination for assimilation (p. 447 A[4]) and if so, there is no doubt but that, the optimal intensity of illumination for assimilation, in the isolated chlorophyll grain, will necessarily be considerably weaker than when it is enclosed in a living cell, forming part of an assimilatory tissue and thus partly shielded from the light.

In the few cases in which Kny and Kolkwitz used pure cultures these were made on gelatine. I have found that such cultures are not nearly as satisfactory as those made on bouillon-agar. In the latter case only, can reliable cultures, of uniform sensitivity at a given age, be obtained, which, when used, do not cause the addition to the fluid employed of any of the waste products of Bacterial action, excepting CO_2, in appreciable amount. The character, sensitivity, and motility, of the cultures, may be markedly affected by the medium on which they are grown, and by the temperature at which they are allowed to develop. (See pp. 365, 434 A[1], p. 555 A[2]). The limits of temperature at which cultures of *B. termo*, to be satisfactory, must be developed, are from 20° C to 25° C.

The waste products evolved by *Bacterium Termo* when in water are innocuous, but the bye products, produced when grown n meat extract, are exceedingly injurious. A moss leaf within a closed cell in *Bacterium* containing water, if exposed to light, remains living for an indefinite length of time, but, if mounted in neutralized meat extract with *Bacteria*, it soon dies. The moss leaf is relatively exceedingly resistant (p. 369, 371 A[1]). The isolated chlorophyll grain, it is hardly necessary to point out, is very much more sensitive.

It may perhaps be as well to mention that it was a considerable time before any definite positive results were obtained, when working on this particular point. Had my own researches been concluded, like those of Kny and Kolkwitz, as soon as the first negative results were obtained, I should perhaps have arrived at the same conclusion as they have done. Fortunately, however, the investigation was persevered with, until it was found, that, under appropriate conditions, with certain plants, positive results could be obtained. To make satisfactory preparations needs some manipulative skill, and the omission of any one of the necessary precautions causes negative or unsatisfactory results to be given. It is hardly necessary to emphasize the fact, that in an investigation of this kind a single positive observation outvalues almost any number of negative ones, especially if the latter are vitiated by errors of experimentation. That isolated chlorophyll grains might possess a power of assimilation was demonstrated to several persons working, at the time, in the Botanisches Institut at Leipzig, including Dr. Klemm, Head assistant and Dr. Richards, now Professor of Botany at Columbia College, New-York. In addition, a formation of minute starch grains in isolated chlorophyll bodies has, in certain cases, been observed, provided the power of evolving oxygen was retained for a sufficient length of time. In darkness these did not appear, and hence, apparently, they were not formed from the sugar solution in which the grains lay.

Kny and Kolkwitz state that experimenting with *Spirogyra*, by means of plasmolysis, treatment with acids etc., it is impossible to cause any stoppage of the power of assimilation without killing the cytoplasm. *Spirogyra* was found at an early stage of my own investigations, to be unsuitable for experimentation in this direction and was therefore not used. In this plant, as in many others, the cell is too sensitive to injurious agencies, and possesses only slight, or no, powers of recovery. It is not impossible that the chloroplastids in this plant are more resistant to injurious agencies than the cytoplasm is. To produce definite results more resistant plants must be employed, and the time during which the injurious agency is acting must be prolonged. This is only possible, when plasmolytic experiments are performed, by accustoming the plants to sugar solutions of successively increasing strengths. Even with the short periods given by Kny, it is interesting to notice, that, in one case, after only 10 minutes

immersal in $30^0/_0$ sugar solution, the power of assimilation did not return until after a period of half an hour, during which time the chloroplastids were unable to assimilate, i. e. were in a condition of assimilatory inhibition, although the cell remained living and finally recovered. This observation coincides fairly well with those made by myself on *Elodea* (p. 435. A[1]), the recovery here taking place, owing to the longer time of immersal, not during the $^1/_2$ hour in $20^0/_0$, partly during the succeeding $^1/_2$ hour in $10^0/_0$, and completely when returned to water.

The inhibition of the power of assimilation, in chlorophyll bodies contained in a living cell, which may persist for a time, after the injurious agency has been removed, is due to a pathological condition, which may be induced in the chloroplastids of many plants by the prolonged operation of almost any injurious agency of sufficient intensity to depress the functional activity of the chlorophyllous cells to the lowest possible ebb, consistent with the preservation of vitality. From this pathological condition recovery may or may not be possible. In the latter case the cell is converted into a non-chlorophyllous one, and yet has been shewn to be capable of remaining living for a week or more (p. 376, 390, 391, 439. A[1]. p. 574. A[2]). In some plants it has been found almost impossible to inhibit the power of assimilation without at the same time killing the cytoplasm. In other plants, the operation of certain injurious agencies only, may cause an after inhibition of the assimilatory power of the chloroplastids, while all other agencies, however applied, cause the death of the cytoplasm before the chloroplastid is markedly affected.

The results of experiments, which are at present in progress, seem to shew that the chloroplastids of mosses and the gonidia of Lichens, exposed to light in an atmosphere deprived of CO_2, retain the power of assimilation as long as the cells remain living, although as has been previously shown (pp. 567—573. A[2]), under such conditions, the chloroplastids of many higher plants speedily lose the power of CO_2-assimilation, though the cytoplasm of the cells in which they lie, is at first living and normal, and may remain so, if the power of recovery has not been lost.

The application of moist heat alone, is, as a general rule, followed, if the cell remain living, by a rapid or immediate return of the power of CO_2-assimilation (p. 386. A[1]), but here also, especially if combined with a certain amount of asphyiation (p. 387 and 388. A[1]) an inhibition of the power of CO_2-assimilation may be produced in living cells.

It is needless to recapitulate further. Full details will be found in the previous publications, in which Kny and Kolkwitz's observations on the rapid return of the power of assimilation to living air dried mosses, and on the presence of a power of assimilation after rotation had been caused to stop, have already been given (pp. 385, 395. A[1]. p. 152. B[3]).

In certain cases Kny and Kolkwitz state that chlorophyllous cells killed by electricity*), chloroform, or nitric acid, continue apparently for a considerable time to assimilate and evolve oxygen. This is hardly surprising, considering that they used impure cultures and unringed preparations. Motile anaerobic bacteria will shew an attraction to, and movement in the neighbourhood of, dying or dead cells for a day or more, in the apparent absence of all external supplies of oxygen. The same is the case with *Bacterium Termo*, if a slight amount of oxygen be allowed to diffuse throughout the fluid in which it lies. The attraction is due to the nutritious substances evolved from the dying cell, and unless a definite attracting cause be present, the *Bacteria* always distribute themselves evenly throughout the enclosed fluid.

Many more or less anaerobic forms when placed in a closed ringed preparation in water, though at first immotile or nearly so, may begin to shew in a short time more or less active movement, i. e., apparently they are immotile when the oxygen partial pressure is high, motile only when it is low.

It is important to remember that the sensitivity of *Bacterium termo* is not always the same. In water it ceases to move when the partial pressure of the enclosed oxygen reaches a certain inhibitory limit. In nutrient solutions the movement continues for a longer time and until a lower partial pressure is reached. On the other hand, *Bacterium Termo*, fresh from agar cultures, requires a higher partial pressure of oxygen to permit of movement, than when it has been kept in pure water for some time. Starvation increases the sensitivity of the *Bacteria* to oxygen. A slight amount of CO_2 increases, a large amount depresses the sensitivity of the *Bacteria* to oxygen and their rapidity of movement. In addition, if a cell is only thinly ringed with vaseline (this was not even considered necessary by Kny and Kolkwitz) a slight amount of oxygen, sufficient to permit of the movement of less strongly aerobic forms than *Bacterium Termo*, and to allow slow rotation to continue in enclosed end-cells of *Chara* kept in darkness, diffuses in. In all such experiments the most careful ringing in necessary (p. 420. A[1]).

Kny and Kolkwitz state, however, that the evolution of oxygen seen by them, tooke place only in light and ceased in

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*) In the case of Spirogyra filaments, killed by the passage of electric currents, one possibility must not be ignored, viz. that the filament may be converted into a secondary battery, the dead cells near the positive electrode being charged with electrolytic oxygen, which may in part slowly be evolved, for a time, after the current has ceased. In the living cell this oxygen combines with the plasma. Hence, asseems to be schewn by experiments at present in progress, the cells near the positive electrode are most markedly affected. Also, using weak currents enduring for a considerable time, the ectoplasmic layer in Nitella may be killed and assimilation cease, although the endoplasmic layer is still shewing slow rotation.

darkness. If so, and if the cytoplasma only had been killed, then here is a case in which the „isolated" Chlorophyll grains continue to assimilate. It must be remembered that it is by no means so easy to be certain that the movement ceases in the dark, as at first sight appears; for, with normal chlorophyllous cells showing active CO_2-assimilation, the evolution of Oxygen and recommencement of movement of the *Bacteria* is almost synchronous with the re-exposure to light, and may follow it with greater rapidity than the eye can be focussed on the preparation, unless several trials are made. In all cases, however, if the evolution of oxygen ceases in darkness and recommences in light, the area of movement can be seen during the first few seconds or first minute to spread out further and further from the source of oxygen. But if the movement, though it appears only to commence as the eye is focussed upon it, is seen to be from the first quite active and to undergo no further perceptible increase, then, it is quite certain, that its apparently instantaneous recommencement is an optical delusion, and that in the darkness it has never really ceased to take place (p. 130. A[3]).

It must also be remembered that under certain circumstances lateral diffusion along a filament may cause a faint evolution of oxygen from a dead cell which is net to a living and activily assimilating one.

A dead wood fibre, mounted in or previously soaked in, meat extract if lying parallel or near to, a green assimilating algal filament, will appear to shew, especially if very sensitive forms are used but also though to a less extent with normal *Bacterium Termo* cultures, an evolution of oxygen which is dependent upon light and ceases in the darkness. This is due to the *Bacteria* when charged with oxygen being attracted by the nutriment to the wood fibre which is permeated with it, and then after returning to the source of oxygen supply, the *Bacterium* is again attracted by the nutriment, and so on. In the absence of oxygen, i. e. in the darkness, the movement and attraction cease. If *Spirillum* forms are enclosed these are repelled from the source of oxygen but not from the dead wood fibre.

It is hence very essential that the *Bacterium* test should always be applied in water, which may contain a trace of salts but no nutriment. If sections of tissues are examined and these become mucilaginous in water, a 2% sugar solution may be used. From the above, the extreme importance of being absolutely certain that the *Bacteria* can obtain supplies of oxygen from no other source but the one in which its evolution is being tested for, is made clear. When relatively large portions of chlorophyllous tissue are being tested, a correspondingly large number of *Bacteria* must be enclosed. Any over-sensitivity of the *Bacteria* employed may be a fruitful source of erron. With over sensitive *Bacteria* an attraction to, and apparent evolution of oxygen from, almost any organic structure, may be detected unless proper precautions are

employed. The repulsion of such forms is here the only safe test for an evolution of oxygen.

There is no à priori reason, why the chloroplastids, in a cell the cytoplasm of which had been killed, might not, especially, bearing in mind the now definitely proved fact, that isolated chloroplastids may continue for a short time to assimilate, also for a time continue, if exposed to light, to evolve oxygen In deed, at one time, it seemed as if certain observations, that Pringsheim and myself had made, pointed to this conclusion. The completed investigation shewed, however, (see p. 415. A[1]. p. 145. A[3]) that whilst an evolution of oxygen might continue in certain cases to take place from a chlorophyllous cell for a short time after its death had occurred, such evolution was, so far as my own observations went, independent of light and, therefore, not a product of a process of CO_2-assimilation. The cases given by Kny may possibly be examples of the continuance of CO_2-assimilation by the chloroplastids, for a short time after the death of the cytoplasm. As shewn above, there is no à priori reason why such should not take place. No results however obtained by means of the *Bacterium* method can be considered as satisfactory unless pure cultures are worked with, adequately closed cell preparations are employed, and full attention is paid to the various special precautions which the researches of Engelmann and myself have shewn to be necessary. Otherwise the use of the *Bacterium* method is more likely to retard than accelerate scientific progress in this direction, namely in elucidating problems connected with CO_2-assimilation.

References.

A[1]. = On Assimilatory Inhibition in Plants. Journal of the Linnean Society. Botany. Vol. XXXI. To 461. 1895. p. 364.
A[2]. = Do. Vol. XXI. To 576. 1896. p. 554.
A[3]. = On the Evolution of Oxygen from Coloured *Bacteria*. Journal of the Linnean Society. Botany. Vol. XXXIII. To 155. 1897. p. 123.
A[4]. = The Effects of Tropical Insolation. Annals of Botany. Vol. XI. No. XLIII. To. 480. p. 439. Sep 1897.
B[3]. = On the Power of with stauding Dessication in Plants. Transaction L'Pool. Biological Society. Vol. XI. To. 159. 1897. p. 151.

23. October 1897.

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The Botanical Society of America. (Bulletin of the Torrey Botanical Club. Vol. XXIV. 1897. No. 9. p. 449—450.)