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THE FLUORESCENT COLORS OF PLANTS[1]

THERE are many fluorescent substances in plants. It was in one of these, quinine, that Sir John Herschel (1845) recognized the peculiar property of emitting "superficial light" of a different character from that transmitted. Twelve years earlier, Sir David Brewster (1833) demolished the Newtonian view of the nature of leaf-green by studying its absorption spectrum. By this means he was led to find that, on observing the light transmitted through increasing densities of chlorophyll, the color of the beam changed from green through yellow and orange to red. "This mode of examining a spectrum by reflection from the particles of a fluid exhibits the phenomenon of opalescence in a very interesting form," he said, adding that he had observed this opalescence or imperfect transparency "almost always in vegetable solutions," and compared it to that in fluorspar, already well known to the mineralogists, and which prompted Sir George Stokes, nineteen years later, to propose, though reluctantly, as giving way to the blandishments of some evil spirit, the term fluorescence. Of the fluorescence of this mineral he remarked, "the brilliant blue of the intromitted pencil is singularly beautiful," and when we look at this specimen before us we can hardly blame him for his rhapsody, nor for his failure to interpret to the satisfaction of later physicists the phenomenon which so intrigued his thought. To grasp clearly the nature of fluorescence was the work of Sir George Stokes, who, my friend and colleague Professor A. S. Eve tells me, was given to working in the "back scullery and a small one at that," using the leaves of laurel and other plants which grew in his garden; and thus was led to the establishment "of a great principle with accommodation and apparatus which would fill the modern scientific man with dismay." This principle is now the food of scientific babes and sucklings, into which happy company we are each of us introduced when we venture into a new field. If their cries are not always intelligible, they at least know what they are after. It will be agreeable, I believe, to recall the experiment which Stokes did, and which gave him the clue to the mystery of fluorescence. It was this. He put some chlorophyll solution into a test tube, and

[1] Address of the vice-president and chairman of Section G—Botany—American Association for the Advancement of Science, Cincinnati, Ohio, December, 1923.

moved it across a spectrum beginning at the red end. "When plunged into the invisible rays, it was certainly a curious sight to see the tube instantly lighted up:[2] it was literally darkness visible. Altogether the phenomenon has something of an unearthly appearance." I have quoted these passages from the writings of Brewster and of Stokes, because their words appear to me to show with what fulness of joy they worked. They seem to have felt that life is more than meat, and the body than raiment.

In order to determine the origin of this curious light, Stokes used a simple but ingenious method of analyzing prismatically the spectrum when reflected by chlorophyll, and by the green leaf, thus obtaining what he called a "derived spectrum."[3]

He found in this way that the fluorescent light is derived by increase of wave length from all the light of wave length shorter than that of the fluorescent light in various amounts. When stated meticulously, this is the Stokes rule. It is with this kind of light that we are now concerned.

Stokes thus settled once for all the fact that chlorophyll in the living leaf is fluorescent. Since then various methods have been used to study the phenomenon in living plants. Simmler (1862), whose curiosity had been prompted by the uncanny illumination which prevails during total solar eclipse, thought to explain the red appearance of foliage when viewed through a filter devised by him as due to the fluorescence of the chlorophyll. Lommel (1871), however, criticized his method, pointing out that the red transmitted by his filters was the portion of the spectrum beyond the fluorescence band of chlorophyll, and devised a filter which would permit only the light lying in this region. With this filter foliage appears black, and so he called his filter, when made up like a pair of dromoscopes (automobile goggles), a melanoscope, in contradistinction to the erythrophytoscope of Simmler.[4]

[2] I have made a transposition of the original text.

[3] The derived spectrum may be seen microscopically by the following means. An Abbe condenser is stopped so as to allow only a marginal cylinder of light to pass through. The parallel beam of a strong illuminant is then directed toward the margin of the mirror so that extreme oblique illumination is achieved. The iris diaphragm is then closed enough to make a slit between its margin and that of the central stop. A spectrum may then be seen in or nearly in the middle field. If a mat of blue green algae (best a red fluorescent species) is then adjusted so that the spectrum falls upon it, the blue-violet-ultraviolet portion is seen to be the seat of red light also.

[4] The point at issue between these authors has lately been of interest in connection with the use of light filters for the detection of camouflage. "Property" forests and coppices when observed through a suitable filter appear red, while green paint, used in imitation of them, appears black. This was achieved independently by Mr. C. F. Stiles, who has afforded me a sample of his filter. This transmits the red beyond $\lambda = 660$. I have found a combination of ruby and violet glass which acts as this, transmitting from 650 on. Simmler's filter (cobalt and iron oxide glasses combined) transmitted the red beyond the B line (foliage appearing red), while Lommel's transmitted the red between B and C (foliage appearing black). I have been able to duplicate Simmler's result but not yet that of Lommel. Lommel's result seems to be due to the reduction in the amount of red light to a narrow band. When I illuminated a Ficus leaf with light devoid of red- yellow- yellow-green, while I could observe a fluorescence red band spectroscopically, the leaf did not appear red to the eye through the Stokes filter, though with stronger light than that used (400 W lamp) it might have. It seems entirely probable that Lommel's contention is correct, namely, that the red appearance of foliage as seen through appropriate filters is due largely to reflected red, but it is also probably reinforced by fluorescent red.

Another method was that of Hagenbach (1874), quite recently used in modified form by K. Stern in his study of fluorescence in *Chlorella*. This consists in projecting upon the material a concentrated beam of light which has been purged of the light from red to yellow-green by a filter, and observing the fluorescent light with any desired means. The spectroscope enables one to analyze it with exactitude. These and similar methods have to do with material in the bulk, while one of the great desiderata of the biologist is to get at appearances in the small. Accordingly, we may now briefly trace the direction of effort to accomplish this.

We owe to the development of general interest in colloidal phenomena the development of the optical means to this end, although such means had originally been achieved in principle much earlier (Siedentopf, 1907). I refer to the arrangement for ultramicroscopy devised by Siedentopf and Szigmondy (1903) foreshadowed by the luminoscope of Tswett (1901) in the matter of orthogonal illumination. This consisted in a light-tight box arranged to permit a beam of strong light to traverse a tube of fluid longitudinally. The contents of the tube were observed laterally. The ultramicroscope was an arrangement for achieving the same purpose with the added high magnification, since a water-immersion lens was used for the lateral observation. In this way suspended particles could be revealed. We well know the purpose this instrument has served in the study of suspensions: Raehlmann (1906) and Gaidukow (1910) used it for the examination of chlorophyll, the latter author, in conjunction with the dark-field condenser, also for studying a variety of plant objects. It yields little to the biologist, however, for it is adapted pri-

marily to the examination of fluids and gases. The needs of the biologist soon led to the construction of the dark field illuminators or mirror-condensers, the prototypes of which were those made in England in the early fifties by Th. Ross, by Wenham and by Stevenson.[5] It is to be noted that they at first made use of the reflected light cone or cone of illumination, a method which did not suit the optical means then available for the examination of very minute objects. Further, with homogeneous immersion the beam passed upward into the objective. In the dark field condensers later devised, namely, at the beginning of the present century, by German manufacturers, a hollow cone of brilliant light of wide angle was obtained, the apex of which, lying between the object slide and cover slip, served to bring the object into view by light which is reflected or refracted into the eye. Much study has been devoted to the optical complexities of the image received (*e.g.*, by Gaidukow). Even diffraction images have their use in interpretation of the object picture.

Among the many purposes to which the dark field condenser has been put is that of seeing and studying fluorescence, especially of chlorophyll. It is to this that our present interest attaches.

Raehlmann (1906) examined chlorophyll[6] and described the suspensoids therein as minutest blood-red particles, resolved by high magnification from a red light cone.[7] He made combinations of chlorophyll and other substances, especially proteins, and speculated on the variety of coloration as possibly due to combinations of this kind.[8]

Gaidukow's studies embraced a wide range of objects, by way of testing out the capacity of the new optical tools found in the various condensers then extant. Among them were a number of plants and chlorophyll. The latter, he found, gave, in alcoholic solution,[9] the red light cone, with no suspensoids, but which appeared on adding water. These he described as red and green, their relative numbers changing with added water till all became colorless. Concerning his observations on plant cells, we note only those observations which bear on the present theme. He examined a series of algae, but only in *Vaucheria* did he observe oil droplets which displayed fluorescent red, though he did not state it thus. For the rest he speaks of seeing white, blue, pale blue-green, red and sealing wax red points in a blue-green *Oscillatoria;* in a violet species, the blue-green points of light were replaced by violet. Somewhat similar results were obtained for *Porphyridium cruentum.* These observations as set down tell us nothing of fluorescence. Certainly Gaidukow did not see general fluorescence in the chromatophores.

At about this time Reichert devised the so-called fluorescence microscope—that is, an arrangement by which objects might be observed when submitted to ultraviolet rays. For this purpose a source rich in these rays, and a train of quartz lenses with appropriate filters were used. Tswett (1911) made the first record of observation of chloroplasts and of *Oscillatoria* which glowed carmine red, the latter inclosed in a pale blue wall. With a spectroscopic ocular he determined the fluorescence spectrum of *Spirogyra* and of *Oscillatoria* (lambda—685–670 and 660–650 for *Spirogyra* and lambda—670–630 for *Oscillatoria*), which would have been more extensive had the full spectrum been used.

Similar observations have been made by Gicklhorn (1914), confirmatory of Gaidukow's work.

The foregoing is approximately the net result up to 1921, when K. Stern published his work on the fluorescence of *Chlorella,* which, however, he studied by the modified Hagenbach method as above mentioned. He applied ultramicroscopy[10] only to solutions and suspensions of chlorophyll, and found himself unable to agree with Raehlmann as to the occurrence of red fluorescent particles. In this I find that I have to support Stern. The red color observed by Raehlmann may have been due to refraction effects, whereby the particles become apparent as red discs at a high focus. Larger suspensoids, or rather emulsoids, procured by preparing emulsions of water and lipoid, appear as minute fluorescent droplets, corresponding to Raehlmann's account. In order to put the matter to test, and having regard also to Stern's work, I prepared a suspension of chlorophyll[11] in alcohol-water, but in no case could I find

[5] For the history of the dark-field illuminator see Locy (1923), Beck (1923) and Siedentopf (1907).

[6] Merck's *chlorophylli puri solutio aquosa.*

[7] Of the Siedentopf and Szigmondy apparatus.

[8] Czapek (Biochemie der Pflanzen 1: 564) appears to have thought that Raehlmann claimed that he saw the fluorescence of chloroplasts ultramicroscopically, but I can not find evidence in the paper cited by Czapek that he made this claim.

[9] Herlitzka (1912) could see no particles proper to phaeophytin-acetone solutions.

[10] Using the Siedentopf and Szigmondy apparatus.

[11] Powder of Saponaria leaves dried 48 hours *in vacuo* at 60 degrees C. was extracted 6 hours with petrolic ether, dried and then extracted 6 hours with ethyl ether. Most of the lipoid had therefore been extracted, but a small amount remained, doubtless. After evaporation, the chlorophyll was taken up with ethyl alcohol, which was mixed with water to make 25, 50 and 75 per cent. mixtures. 25 per cent. water: suspensoids small, uniform, appearing white in apex of inverted light cone; 50 per cent: suspensoids somewhat larger white, fluorescent in ultraviolet; 75 per cent. water: flocculation, the small flocks green, suspensoids white, fluorescent in ultraviolet.

any evidence of red color proper to the suspensoids. Nevertheless, these suspensoids are fluorescent.

Stern was able to see that *Chlorella,* in bulk suspensions of pure culture, is fluorescent when illuminated by light passed through a filter admitting light between E and H only. By comparing the fluorescence of various chlorophyll mixtures, he found that only those into which lipoid has been introduced showed fluorescence. Without tracing his argument, he concluded that only molecularly dispersed chlorophyll is fluorescent, and that colloidal or solid chlorophyll is not. Since chlorophyll in the chloroplast (as in *Chlorella*) is fluorescent, it is therefore molecularly dispersed, though it may be in a viscous solvent (lipoid) itself colloidally dispersed.[12]

We have thus indicated the net result from the use of microscopic means, which as late as 1921 had yielded very little. Were it possible to see the fluorescence of chlorophyll and other pigments microscopically and at the same time the structures involved, it would give us an additional means of investigation. Such means we now have in the dark field illuminator of wide aperture, when used in the manner which I have already described (1923). The method consists in viewing the object at the apex of the light cone after it has been reflected from the cover-glass.[13] To this end are required a thin object slide and a dry objective supplied with an inside stop, the latter to obviate the flare, which is present, though to less extent than with an oil immersion.

By this means we obtain, in general, a softer illumination, albeit brilliant enough for very many purposes, and a completely black field. But when fluorescent organisms (especially those containing the water soluble pigments, phycocyanin and phycoerythrin, namely, the blue-green algae, some diatoms, florideae, etc.), are viewed, a most remarkably beautiful scene is produced, one which has "something of an unearthly appearance," to quote Stokes's words again. I show you a series of reproductions of organisms made by color-process photography[14] which, striking as I venture to believe they are, do the original subjects scant justice, as you will see on examining the preparations which are ready for your inspection.

It will have been observed that the dominating light received by the eye is that of fluorescence. This is more or less so according to the technique of preparation and of optical manipulation. Inevitably, when the entire spectrum is used, the total illumination consists of waves lengths of all dimensions, so that the spectrum obtained (with a spectroscopic ocular) would not be a pure fluorescence spectrum. In order to prove that the dominant color seen is that of fluorescence, I have photographed *Oscillatoria sancta* illuminated with light passed through a filter (methylene blue) which admitted light $= \lambda$ 520 to 400 and 750 to 720 only. The image was passed through a yellow filter transmitting only waves lengths longer than 560, when it has much the same appearance to the eye as when seen in the fluorescence microscope. Having determined spectroscopically that the light thus to be received by the negative plate was embraced between 580 and 560 (approx.), this being the fluorescence spectrum of the organism as thus illuminated (and which corresponds fairly closely with the results of Tswett, 1911, and with the fluorescence spectrum of the extracted phycoerythrine [$\lambda = 550$ to 670] the plate was exposed for five hours, with the result which is shown you. The photograph of *Spirogyra* shown was made in similar fashion but with eleven and one half hours' exposure. You will observe that no trace of reflection from cell-walls or other bodies is visible, as is the case if the photograph is made with full illumination, though using only an ocular filter admitting only red. Here, as you see by the photograph, we get reflected red. In one negative bacteria may be seen thus illuminated, but there is little doubt that this color is reflected fluorescence red.

It should be noted that the colors achieved by color process photography do not correspond with more than loose approximation to the colors which emanate from the objects, and that one can just as easily prove that an object is green as that it is red, by the particular method which I have employed. You are, therefore, asked to add a measure of faith to sight in beholding these photographs. As a rock on which faith may rest, I show you the spectra in diagram, from which it will be seen that the fluorescence color of *Spirogyra* should be a deep red, while that of phycoerythrin should be a yellow-red or orange.

The advantage of this method of using the dark-field illuminator lies, however, not merely in rendering visible the fluorescence in the organism—this it does only with difficulty for chloroplasts, which demand a special technique, but in rendering visible structure at the same time. The former purpose is well served by the fluorescence microscope, which, however, does not lend itself to the study of structure. Objects thus seen have, moreover, low visibility. I have also had some opportunity of using the mercury-vapor lamp, the light being served to the object through a quartz dark field illuminator. This

[12] Space limits command us to refrain from entering upon the much discussed problem here indicated, that of the condition of chlorophyll in the living chloroplast.

[13] Thus returning to the earlier method of Ross and of Wenham.

[14] Including various species of *Chroococcus, Oscillatoria, Nostoc, Arthrospira* (3 spp.), *Cylindrospermum, Chantransia, Spirogyra, Pleurococcus,* etc. The Paget method was used.

has no advantage for achieving the results described over a 400 watt filament lamp or a 4 ampere arc, though with suitable filters it will be very useful for a spectrocopic study of living organisms.

It should further be remarked that the failure to observe fluorescence in organisms under the ordinary conditions of dark field illumination lies in part in the fluorescence-dispelling power of reflecting surfaces, and it is at the apex of the upright cone of light that this is at the maximum. For this reason, also, one employs mounting media which obviate these surfaces as far as possible. (Glycerine, cane-sugar solution, etc.) Nevertheless, even with homogeneous immersion lenses one can see fluorescence, particularly when one has learned its appearance. As has often been observed, solutions of fluorescent substances (chlorophyll, eosin, etc.) are readily seen to be fluorescent.

In the above regard, the chloroplast offers considerable difficulty, partly because of the very low visibility of the fluorescence (it being dark red) and partly because of granules, the nature of which we are in doubt. I illustrate these facts with a plate of chlorophyll in balsam and with one in collodion, with and without the addition of a small amount of oil. You will observe that the visibility of the fluorescence is at once dispelled when one backs these plates with one of ground glass. And when a black velvet disc with white spots is so placed, the fluorescence is dispelled by the white spots, which now appear green. It seems probable that the green granules which are to be seen in chloroplasts, the whole stroma of which appears fluorescent, are really colorless, appearing white, just as cyanophycin granules do.

We may now pass on to consider the results which have accrued from the use of the method, avoiding too much detail. Fluorescence has been observed in a number of bacteria (casual observations), in all the species (about 20) of the blue-green algae examined with one notable exception of an apparently undescribed species, in some desmids, in several species of *Spirogyra,* in *Hydrodictyon,* in several of the *Protococcales* (*Gloeocystis, Kirschneriella, Excentrosphaeria*), in *Gloeocystis,* in *Vaucheria,* in several diatoms (*Navicula, Meridion, Bacillaria,* etc.), and in the fresh-water floridean, *Chantransia violacea aff.* and finally in the chloroplasts of a number of higher forms.

A feature of the blue-greens which stands out is the variety of nuance. In general, the blue-green species which contain phycocyanin are fluorescent red, while the brownish or violet tinged kinds containing phycoerythrin are orange fluorescent. Boresch (1921) found an alignment of species, 22 in number, in accordance with this. But I have observed that in either group there are less striking but still obvious shades of color suggesting mixtures, from almost if not quite pure yellow to deep carmine red. One explanation obviously may be found in the admixture of light due to reflecting surfaces, a matter which has been discussed since 1883 (Reinke), and as I have experimentally illustrated. That this is the case in the blue-greens is shown by the fact that cells of some species of *Oscillatoria* which have been shrunken by cane sugar or glycerine appear deep red, but as the shrinkage disappears by taking up water, they become orange which appears deeper where there are few granules and light where there are many, as near the transverse walls. Another explanation may be found, possibly, in the modifying effect of one fluorescent substance on another, illustrated when I mix alcoholic solutions of fluoresceine and chlorophyll. I am not altogether convinced that the blue and red pigments do not both occur in the same species, some observations suggesting this. At all events, in nearly all cases we have to reckon with the presence of both chlorophyll and of one of the water-soluble pigments.

As to the way in which the pigments occur, I have already advanced evidence for believing that the phycocyanin (or phycoerythrine) is confined to numbers of minute vacuoles. It is very difficult and often impossible to resolve them except in very large cells, such as the spores of *Cylindrospermum,* in which they may be identified—their fluorescent contents also—with the oil immersion. They seem not to be confined to the so-called chromatophore, but there is a difficulty of observation of this relation as also of the limits of the vacuoles, arising from the masking of finer structure by the fluorescent pigment. This has suggested the importance of the use of ocular filters, as did Timiriazeff (1904) in observing chlorophyll bodies. Photographers have found out that this method enables them to get results otherwise impossible, and microscopists will profit by the same method.

In the blue-green algae[15] phycoerythrin does not seem to occur in this way, but rather as in the Florideae, in which it is confined within the chloroplasts. In these it is coterminous with the chlorophyll and with the limits of the chloroplast. Its water solubility suggests that the relation of the pigment to the plastid is an adsorption one. It is more difficult to decide about chlorophyll, even assuming that the above is true. Its lipoid solubility, and its index of fluorescence in various water-lipoid mixtures have led Stern to argue strongly for the view that it occurs in the chloroplast dissolved in lipoid, the lipoid itself, it may be, being dispersed.

[15] We are accustomed to this adjective, but it would be useful to use red-green to distinguish those species containing phycoerythrin.

The above statements are avowedly subject to revision; nevertheless, it may be confidently hoped that the method under discussion will prove of material assistance in the solution of this problem.

A further word on the differences of fluorescence color. I have repeatedly observed that two species almost identical, but, I suppose, not entirely so, differ in fluorescence colors, irrespective of the genus. In this connection I was interested to note that Teodoresco found in Bucharest *Nostoc commune* containing phycoerythrin, whereas other examples of this species contain phycocyanin (as does a sample I have from China). These may be examples of mutability of color as observed by Gaidukow (1902), but I incline to think not, and that these are different organisms.

The bizarre color effects observed by Gaidukow by means of the upright light cone I have also seen, but they may also be seen in nematodes which so frequently occur with blue-greens. One is tempted to revert to the theory of generation of a pre-Redian age and to believe that these pests are derived from filaments of *Oscillatoria,* or to attach little importance to mere color appearances, many of which are refraction effects. The extruded white cyanophycin granules which look very convincingly red at a high focus, appear blue *in situ* in red fluorescent species because seen through a screen of blue pigment as already pointed out. It is, as a matter of fact, very difficult to ascribe a particular color to minute granules when seen with dark field illumination, due account of which has been taken before voicing any of the preceding statements.

The heterocyst in no case observed contains any fluorescent pigment.

Of three species of *Arthrospira,* found in material sent to me by Professor Faull from Toronto, one is red-, one orange-fluorescent and one, greenish yellow by transmitted light, is beautifully opalescent blue when viewed with the dark field illuminator (photograph), but contains no fluorescent pigment, it being quite invisible in pure ultraviolet (fluorescence microscope). While slender, it is a striking species,[16] sharply contrasting with the fluorescent[17] species in the wide amplitude of the spirals. As you see in the photograph, cyanophycin granules appear white, as they do also in *Chroococcus* (slides).

When the blue-greens are heated enough to destroy the water soluble pigment, they become green. The chlorophyll now not being masked, the fluorescence spectrum may be seen with the microspectroscope, using the inverted light cone, and the appropriate filters. It lies between 650 and 700.

Aside from the blue granules (cyanophycin) of the deep-red fluorescent species, there are visible other granules. These are perhaps the gas vacuoles according to Klebahn (1922). They shine very brilliantly, as do also entire cells, which, however, appear to be dead. I have observed similar granules extruded from chloroplasts, while sometimes yellow granules have been seen (*Aspidistra*).

After lying in glycerine for some time, varying with the species, the water soluble pigment leaches out, and its fluorescence may then be seen in the apex of the light cone. In this way, with even a very small amount of material, the pigment may be identified, though there may be insufficient to give a color by transmitted light.

Diatomaceae: The difficulty of observation encountered by Gaidukow (1910), *viz.,* the masking of the interior coloration by the shell, is in many cases quite overcome by using the inverted light cone, though not always or not entirely. Furthermore, the chloroplasts themselves afford reflecting surfaces which have the same effect. Since Gaidukow failed to observe anything, either of structure or coloration, the following observations indicate a distinct advance.

The diatoms fall into two groups so far as I have been able to study them. In one a fluorescent pigment occurs in solution in the vacuoles; inasmuch as these vacuoles are filled with an oil, which is lightly colored yellowish green by transmitted light, it may be inferred that the pigment affording the deep red fluorescence is in solution in the oil. The chlorophyll in these forms is not simultaneously observable as fluorescent, because of reflections. Particularly beautiful is *Meridion,* each cell of which contains a large and a smaller vacuole appearing to be set as gems in a golden-green setting.

Other species appear to contain no oil vacuoles, and the fluorescence is confined to the chloroplasts. These forms contain two fluorescent pigments, chlorophyll and a water-soluble red fluorescent pigment.[18] Upon heating, the latter disappears, and the chlorophyll, being demasked (Kohl, 1906), now appears as brilliant green granules. Here, as in the blue-green algae, there has probably occurred a disturbance of the chlorophyll colloidal complex, whereby the fluorescence is rendered invisible. The fluorescent color of these forms in the living condition is deep blood-red.

Spirogyra: This and other forms containing presumably only chlorophyll by way of fluorescent pigment are by no means as easily seen to be fluorescent

[16] Distance between turns of spiral 17 microns, width of spiral 19 microns, of cell 3.4 microns, the individual approaching 1 mm in length.

[17] Bright green (transmitted light) with large bright granules; width of spirals 12–13.6 microns, distance between turns 17 microns.

[18] This may be carotin, according to Kohl's (1906) view.

as those containing water-soluble fluorescent pigments. The difficulty arises again from the multitude of reflecting surfaces. Accordingly, the smaller and more delicate species (*S. porticalis,* e.g.) containing one or two chloroplasts and few granules are the best for observation. The most successful preparations have been made in cane sugar solution, which of course causes the cells to collapse. This very result, however, enhances the observability *per oculis* of the fluorescence. When seen, the chlorophyll band appears somewhat various in color, this resulting from the unavoidable mixture of reflected with fluorescent light. In its purity the latter is very deep blood-red, especially when seen in a chloroplast which lies athwart the axis of vision. This color varies from blood-red to brilliant vermilion more or less masked by the light reflected from granules. The lenticular body of the pyrenoid is not fluorescent. Judging from the transmitted light appearance we should expect that the globoidal mass within the lens would be fluorescent, and this sometimes may be seen, but not constantly.

On heating to boiling point, the chlorophyll is not rendered non-fluorescent, but it becomes more or less extruded into vacuoles which originate in the chloroplast, and these now contain the chlorophyll, or at least a derivative of it, which can be seen to be fluorescent without the aid of filters. In some species, however, filters are a necessary aid to observation.

Chantransia possesses a very pronounced orange fluorescence more or less irregularly red, especially in the spores. The chloroplasts are irregular bands having an olive-green transmitted light color. When first observed with the inverted light cone the fluorescence can be seen to be confined strictly to the chloroplasts. In the course of a few minutes, however, the light, probably by the heat engendered, causes the chloroplasts to lose their form so that the whole cell becomes filled with fluorescent pigment. Similarly, the plant becomes green upon heating and the yellow fluorescent pigment destroyed.

The relation of the pigment above described which conforms, of course, with previous knowledge, is seen most beautifully in germinating spores, as during germination there remain only a few small chloroplasts in the original spore cell.

Chloroplasts of Higher Plants: The treatment of chloroplasts with glycerine or cane sugar is necessary to keep them from disintegration which, however, appears not to proceed in water so uniformly nor is so rigidly conditioned by structure as has been thought by Timiriazeff and by Wager. In water or in very weak glycerine the chloroplasts take up water and form large vacuoles devoid of fluorescence, while in strong glycerine or cane sugar the chloroplasts appear to suffer shrinkage accompanied by extrusion of fluorescent drops, the brilliant granules which may always be observed remaining intact.

The observability of fluorescence by the means which I have described is nearly always more or less impeded by the presence of these brilliant granules which appear green. I have been inclined to believe from this observation that the chlorophyll occurs in the chloroplasts in two conditions, a suggestion already made by Stern. Since, however, the granules on extrusion from the stroma are white, we believe that their green appearance when seen *in situ* is due to the transmitted light color of chlorophyll in the surrounding stroma, and that therefore the granules do not contain chlorophyll. The fluorescent light emitted by the chloroplast is coterminous with the non-granular stroma. It is, therefore, rather easy to acquiesce with the somewhat generally accepted view that the chlorophyll in the chloroplast is associated with lipoid. The occurrence of fluorescent pigment in large oil drops requires explanation. Gaidukow observed these in *Vaucheria.* Whether these oil drops may be extruded as assimilatory substance either as a normal procedure or as the result of the contraction of the chloroplast by the glycerine or cane sugar, as the case may be, is still an open question. Upon mechanical disturbance these oil drops run together and become very conspicuous in the field, and have much the appearance of the drops of fluorescent substance derived from the blue-green algae when treated with cane sugar. It is quite possible, of course, that the pigment in the oil of *Vaucheria* is not chlorophyll at all, but may be similar to or identical with the pigment in the vacuoles of the diatoms. This idea is supported by the observation that one of the unicellular green algae, *Gloeocystis vesiculosa* (as seen in the photograph before you), which contains a red fluorescent pigment in the vacuole while, simultaneously, the chlorophyll does not appear fluorescent. In *Pleurococcus* this pigment extraneous from the chloroplast does not occur. Whether *Chlorella* studied by Stern conforms to this observation would be worth while to know in view of the observations and conclusions of that author.

In concluding, I may remark that this method of microscopic observation will most certainly add much interest to the study of green plants, and this, as I think you will agree when you have examined the preparations awaiting your pleasure, because of the astonishing and altogether remarkably beautiful pictures which these organisms, when illuminated by their own fluorescent light, afford. This interest is measurably enhanced on the reflection that the fluorescent pigments involved may all prove to be of much greater importance than at present supposed, chlorophyll excepted from the supposition. It would be leading us too far afield to consider, in this con-

nection, the possible significance of the physiological studies in the realm of photodynamics and their bearing on the nature of the chlorophyll mechanism,[19] but the opinion may be ventured that further attack, in which the water-soluble fluorescent pigments receive more extended and more critical study than they have as yet, will bring a rich victory.

Nor can one be aware of the discussions in the field of phototherapy, instancing, for example, the remarkable curative effect of light in rickets, without feeling that there exists some relation between this and the fluorescence of the blood pigment.

It may be added, also, that the taxonomist will find it to profit him to make use of the method for the finding and identification of the blue-greens. It is quite surprising with what ease one can find the organisms. For searching purposes alone, it is incomparable. Added to this is the fact that these organisms afford nuances of color which permit closely similar organisms to be separated and identified much more readily than when transmitted light alone is used. Furthermore, many of the grass-greens will be found to contain other fluorescent pigment than chlorophyll, and we are not compelled to await the outcome of the more tedious and time-consuming methods of the pure-culturist and biochemist, for the discovery of such organisms. We can now find the organisms by optical search, and, then, if it is desired, employ those methods with more direction. As I have already shown, too, there may be many organisms which do not contain fluorescent pigments, but which have not hitherto been regarded as members of the *Bacteriaceae* and perhaps which should not be so regarded, but which occupy a sort of intermediate position between these and the blue-greens. Other possibilities present themselves, but space prevents further discussion.

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[19] Discussions occur in Gicklhorn's 1914 paper and in Grafe's recent book, Chemie der Pflanzenzelle.

THE ECONOMIC VALUE OF GOVERNMENTAL SCIENTIFIC WORK[1]

THE subject that I have been asked to speak on is an interesting one to other persons than those who devote their lives to science. The economic value of the scientific work of the government is to some extent appreciated by the people of the country; I think, exactly to the extent that they come into contact with it and know about it. Perhaps, however, they do not realize that it is scientific work. There are various organizations of the government that perform services for the people, which services are based on scientific research—sometimes on very definite and exact knowledge, sometimes only on whatever state of knowledge has been reached after research has been pursued as far as it has gone, up to the present time. The people know the service they are getting. The western farmer knows something about the Bureau of Animal Industry that cures hog cholera, but it seems to me that the farmer does not think of it as scientific work; he thinks of it as a very valuable service which means dollars and cents to him. The southern cotton planter knows of the Bureau of Plant Industry and its progress in ridding him of the thing that threatens his welfare—the boll weevil. He may know that it is the result of scientific investigation, but he is more likely to think of it as a certain service that he needs on his plantation. Every shipper of meat in summer wants to know whether there is a hot wave coming that will require him to put more ice in his refrigerating cars. He depends on the Weather Bureau to give him that information. This is a service that is performed by the Weather Bureau and received by the people who hardly think of it as scientific work at all. Everybody knows that there is scientific work and that the government does it, but it is the idea of service that is most prominent in the minds of the people that receive it. The warnings that keep vessels in harbor when dangerous storms are coming are given out by the same bureau; warnings of frost that make fruit growers get their petroleum heaters out into the orchards. I shall not undertake to go through the various services performed by the Department of Agriculture, and I could not at all give a complete list of the services performed by the Bureau of Standards; I speak of certain things that are generally known. The farmers know certain scientific work of the Department of Agriculture and the manufacturers

[1] Address made at the dinner of the American Geophysical Union at its annual meeting.