INTERNAL CONVERSION IN THE PHOTOSYNTHETIC MECHANISM OF BLUE-GREEN ALGAE

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INTRODUCTION

All plants and bacteria that do photosynthesis contain one or more of the chlorophylls. The wave length dependence of photosynthesis is, at least in the red end of the spectrum, very similar to the absorption spectrum of the chlorophyll present. In a plant like *Chlorella* of all the substances present only chlorophyll is "black" enough to absorb the energy needed to maintain the observed rate of photosynthesis in red light. These three considerations make it certain that chlorophyll plays a major role in photosynthesis.

In addition to chlorophyll all the photosynthetic organisms so far studied have been found to contain other colored substances such as carotene, xanthophyll, phycocyanin, phycoerythrin etc. The question whether or not any of the light energy absorbed by these accessory pigments is used by the plant to reduce carbon dioxide has interested man since the time of Engelmann (1883).

During the last fifteen years there has been published a series of experiments that give definite answers to the question for several of the pigments. Roelofsen (1935) and French (1937) have shown that in the case of several strains of purple bacteria the light energy absorbed by the red pigments is not used for photosynthesis. Dutton and Manning (1941) using the diatom *Nitzschia closterium* found that light absorbed by some or all the carotenoid pigments can be utilized in photosynthesis. Emerson and Lewis (1942) showed that light absorbed by phycocyanin in *Chroococcus* is used in the process of photosynthesis with an efficiency almost as high as if it had been absorbed by chlorophyll, while the light absorbed by the carotenoid pigments is for the most part unavailable for photosynthesis. It is the purpose of the present paper to point out a mechanism of energy transfer from phycocyanin to chlorophyll, the efficiency of which seems to be high enough to account for the results of Emerson and Lewis. This new process is, except for the scale, identical with the process of internal conversion that we have in the study of radioactivity.

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The Efficiency of Phycocyanin

From Fig. 4 in the paper of Emerson and Lewis we can calculate values for the quantum yield for both chlorophyll and phycocyanin. The quantum yield is the number of carbon dioxide molecules reduced per absorbed quantum. We let ϕ_o be the value for chlorophyll and ϕ_d be the value for phycocyanin. We assume that both are independent of the wave length. At 676 mμ, where chlorophyll absorbs 94 per cent and phycocyanin 6 per cent, the quantum yield is found to be 0.085. In the region 560 to 640 mμ, in which phycocyanin absorbed 80 per cent and chlorophyll only 20 per cent, we have about 0.078 as the average value. The two equations

$$0.085 = 0.94\phi_o + 0.06\phi_d$$

$$0.078 = 0.20\phi_o + 0.80\phi_d$$

give

$$\phi_o = 0.0856$$

$$\phi_d = 0.0761 \qquad \frac{\phi_d}{\phi_o} = 0.89.$$

Due to the difficulty in estimating the exact fraction of the light absorbed by the separate pigments the above values do not have very great precision. However they do show that any attempt to explain the phycocyanin photosynthesis by a transfer of the energy to chlorophyll must make use of an energy transfer system that has an efficiency in the neighborhood of 90 per cent.

THEORETICAL

In describing the mechanisms by which energy may be transferred from one atomic system to another, we may either appropriate approximate descriptions which have been found useful in one or another atomic phenomenon, or alternatively try to classify these mechanisms in terms of the nature of the electromagnetic fields involved. Three familiar mechanisms that are naturally suggested for the energy transfer from fluorescent dyes (phycocyanin) to chlorophyll are (1) collisions of the second kind, involving the transfer of energy from one to another molecule on collision; (2) emission of radiation from one molecule and its reabsorption by another; (3) internal conversion, or the resonance transfer of energy from one oscillator to another in resonance with it, and lying within the quasistatic rather than the wave zone field of the former. The first of these will predominate when the two atomic systems are separated, during the transfer, by distances of the order of atomic dimensions, and will not depend critically on the existence of resonance between the two; the second will depend critically on resonance, and will operate when the distances are far larger, of the order of many wave lengths corresponding to the atomic periods; the third is intermediate, and operates when the distance is large compared to

atomic dimensions but small compared to a wave length. It will be clear from this that the distinction between the three mechanisms is not absolute, but that in any given problem one effect or another may greatly predominate.

It is easy to see that in the dye-chlorophyll transfers (*d-c* transfers), only the third mechanism can be important, as one might indeed expect from a rough estimate of the average separation of dye and chlorophyll oscillators, of the order perhaps of some 10^{-7} to 10^{-6} cm. The striking thing about these transfers is their extreme efficiency; more than 90 per cent of the light absorbed by the dye would seem to find its way to the chlorophyll. It is clear that such efficiency could not be expected from the random collisions between two quite rare molecules in a condensed medium; by far the greater part of the energy in the dye would be lost to other molecules and dissipated as heat before a collision effective for transfer could take place. This is the more patently true because in some cases dye and chlorophyll are not even soluble in the same phases, a circumstance which would effectively prevent any collisions at all from taking place, and which also, taken in conjunction with the slight changes in absorption spectrum both of dye and chlorophyll, argues against the possibility of an appreciable chemical binding of the two molecules which would permanently keep the corresponding oscillators in atomic contact. In fact a rough estimate of the characteristic time for transfer by collisions of the second kind gives about 10 microseconds, a time long not only compared to that for the dissipation of energy in heat, but also for the loss of energy by fluorescence of the dye.

Equally clear is the fact that the emission of fluorescent radiation and its reabsorption by the chlorophyll will not give an adequate transfer; the percentage so transferred is roughly

$$F\sigma nR \tag{1}$$

where F is the fluorescence yield of the dye, σ an average adsorption cross-section per molecule of chlorophyll averaged over the fluorescent spectrum, n the concentration of chlorophyll in the cell, and R the dimensions of the cell. From (1) we get only about 10^{-3} of the observed transfer.

We have, however, to remember that the transfer through time-varying electromagnetic fields will be enormously enhanced if the absorbing oscillator lies within a distance small compared to a wave length of the emitter. This is just the effect which accounts for the very large photoelectric absorption of nuclear gamma rays by the tightly bound electrons in the surrounding atom, an absorption which here too exceeds by an enormous factor that computed from (1). In fact the *d-c* transfer is altogether analogous to this internal conversion of gamma rays; both the separation of emitter from absorber and the wave length of the radiation are multiplied by a factor of some 10^4. The reason for the enhanced transfer is, of course, that the electric field of an oscillator (emitting primarily electric dipole radiation), which in the wave zone falls off linearly

with the inverse distance from the emitter, increases, as we approach the emitter within distances small compared to a wave length, like the inverse cube of the distance.

We can readily calculate the ratio of the *d-c* transfer, for given separation of the *c* and *d* oscillators, to the actual fluorescent yield *in vivo*. This latter yield may, of course, be very much smaller than the *in vitro* fluorescent yield, because the excitation energy of the oscillator is drawn off by the *d-c* transfer before it can be radiated. On the other hand the sum of the *d-c* transfer and *in vivo* fluorescent yield can be much greater than the *in vitro* fluorescent yield, because energy in the *d* oscillator may be drawn off by the *d-c* transfer so rapidly that its loss to other degrees of freedom in the dye molecule and subsequent conversion to heat may be greatly reduced. Thus we need not be troubled by the fact that the *d-c* transfer characteristically exceeds the *in vitro* fluorescent yield, nor that the fluorescent yield is itself characteristically depressed by the *d-c* transfer in the cell.

To carry out the calculation we suppose that the *d* oscillator is described, outside the oscillating changes of the dye, by the electromagnetic potentials

$$\vec{A} = \frac{\vec{2a}}{r}\cos 2\pi\nu\left(t - \frac{r}{C_1}\right) \tag{2}$$

$$\phi = \frac{2(\vec{r}\cdot\vec{a})}{r^2}\left[\cos 2\pi\nu\left(t - \frac{r}{C_1}\right) + \frac{C_1}{2\pi\nu r}\sin 2\pi\nu\left(t - \frac{r}{C_1}\right)\right]$$

with C_1 the velocity of light in the medium. Here we need consider only one oscillator frequency ν. The quantity $\vec{a}$ measures the amplitude of the oscillation, and may be evaluated from the actual fluorescent yield F *in vivo* by calsulating the integral of the Poynting vector for the field (2) over a large sphere surrounding the *d* oscillator, and dividing the result by the quantum energy; this gives:

$$F = \frac{16\pi^2\nu\vec{a}^2}{3hC_1} \tag{3}$$

For distances small compared to a wave length, (2) gives an electric field

$$\vec{E}_e = \frac{2C_1\sin 2\pi\nu t}{2\pi\nu r^3}\left[\vec{a} - \frac{3(\vec{a}\cdot\vec{r})\vec{r}}{r^2}\right]. \tag{4}$$

If an oscillator of frequency ν is situated at a point $\vec{r}$ it will absorb quanta from the field (4) at the rate

$$\frac{C_1^2\vec{M}^2(\vec{a}^2 + 3(\vec{r}\cdot\vec{a})^2/r^2)}{3h^2\nu^2r^6} \tag{5}$$

Here $\vec{M}$ is the matrix element of the electric moment corresponding to this absorbing oscillator, and is connected with the absorption coefficient for light of frequency ν by the relation

$$\sigma = \frac{8\pi^3 \vec{M}^2 \nu}{3hC_1} \quad \text{or} \quad \vec{M}^2 = \frac{3hC_1\sigma}{8\pi^3\nu}. \tag{6}$$

Thus, on averaging over angles, we find that the rate at which quanta are transferred from dye to chlorophyll, when the separation of the two oscillators is r is just

$$T(r) = \frac{\vec{\sigma a^2 C_1^3}}{4\pi^3 h\nu^3 r^6}. \tag{7}$$

It will be seen that this quantity increases rapidly with decreasing distance; and if for orientation we suppose that the oscillators are uniformly distributed with density n outside a sphere of radius d, we get

$$T = \int_{r>d} nT(r)\, dV = \frac{\vec{\sigma a^2 C_1^3 n}}{3\pi^2\nu^2 h d^3} \tag{8}$$

Or, for the ratio of d-c transfers to fluorescent yield

$$\rho = \frac{T}{F} = \frac{\sigma n \lambda\!\!\!^{-4}}{d^3} \quad \text{with} \quad \lambda\!\!\!^{-} = \frac{\lambda}{2\pi} = \frac{C_1}{2\pi\nu} \tag{9}$$

The wave length should of course be evaluated for the medium water, and not for air; *i.e.* $C_1 = \frac{C}{\mu}$, with μ the index of refraction.

The quantity d, which enters into the result (9) so decisively, cannot be estimated *a priori* with any great accuracy, but we can find an argument to fix a lower limit for it, and one to fix an upper limit. On the one hand we know that both c and d oscillators are imbedded in extremely complex and quite large molecules, and a lower limit on d is thus the sum of the radii of these molecules, for which we may take

$$\tfrac{1}{2} \times 10^{-8}(\sqrt[3]{M_c} + \sqrt[3]{M_d}) \text{ cm.} \tag{10}$$

where M_c and M_d are the molecular weights of the two molecules. The lower limit gives

$$\mathrm{d} > 10^{-7} \text{ cm.} \tag{11}$$

and will be substantially too low if the two molecules are necessarily dispersed in different media, so that the thickness of the boundary layer would have to be added to (10). On the other hand it would hardly be expected that the d could

exceed the maximum distance of a point in the cell from a completely uniformly distributed chlorophyll, which gives

$$d < d_o; \qquad d_o = \frac{\sqrt[3]{3}}{2} n^{-\frac{1}{3}} \tag{12}$$

$$d < 5 \times 10^{-7} \text{ cm.}$$

It has thus to be demanded that in general the experimental values of the ratio (9) correspond to a d lying within the limits (11) and (12). It would seem that this is the case.

Finally we may remark that the rate of *d-c* transfer given by (8) is of the order 10^{11} per second, and thus high enough to cut down very considerably the transfer of oscillator energy to other degrees of freedom in the dye, and thus to reduce the fraction of energy dissipated as heat.

EXPERIMENTAL

Chroococcus was grown in the manner described by Emerson and Lewis and the various data needed to test the equations proposed above were determined.

Microscopic examination of the cells showed them to be spherical with an average diameter between 1.8 and 2.5 μ. They were surrounded by a gelatinous sheath about 1 μ thick. Inside the cell, so far as can be seen by the microscope, there was a uniform solution of chlorophyll and phycocyanin.

Two 15 cc. samples of a suspension were centrifuged down and the chlorophyll extracted with methyl alcohol. Chlorophyll determinations were then made by measuring the extinction coefficient for the 6678 Å line of helium and using as a standard the value of 0.454 for a solution containing 10 mg. of chlorophyll per liter of methyl alcohol as given by Arnold and Kohn (1934). The two determinations gave 1.65×10^{-8} and 1.60×10^{-8} mols of chlorophyll per cc. of the original suspension. A third sample of the suspension was taken to make a cell count in a Petroff-Hausser slide. This gave $1.2 \times 10^{8} \pm 6$ per cent cells per cc. of our original suspension. Thus we have

$$\frac{\text{Chlorophyll molecules}}{\text{in average cell}} = \frac{1.62 \times 10^{-8} \times 6 \times 10^{23}}{1.2 \times 10^{8}} = 8.1 \times 10^{7}.$$

From the diameters given above we can calculate that the volume of a single cell is between 3×10^{-12} and 8.2×10^{-12} cc. and thus that n, the number of chlorophyll molecules per cc. inside the cell, is between 1×10^{19} and 2.7×10^{19}.

Chroococcus cells are unique among microorganisms for the ease with which the cell wall can be broken down and the juices obtained cell-free. A suspension that had been centrifuged to remove the culture medium, then resuspended in a small amount of distilled water, was put into a glass 10 cc. hypodermic syringe that had the tip sealed. The barrel of the syringe was slipped into a short length of rubber vacuum tubing and then clamped in the jaws of the chuck on the lathe. The dead center in the tail stock was replaced by a large rubber stopper. With the lathe turning about once per second, the plunger was kept from rotating with the left hand while the right

was used to turn the tail stock handwheel so as to drive the plunger into the syringe. Thus the suspension was forced out in a spiral path between the plunger and the barrel. The material was collected in a small beaker as it dripped off the back end of the glass barrel. This method, which had been suggested to us by Dr. French, will open 98 to 99 per cent of the *Chroococcus* cells in one passage provided the syringe has been used only once or twice before. A syringe that has been used a number of times becomes very ineffectual probably due to the glass surfaces becoming smoother with use. (Incidentally, *Chlorella* cells cannot be used, as some material in these cells will cause the two glass parts to "freeze" together.) One further centrifugation of the material obtained gave a cell-free solution of chlorophyll-protein and phycocyanin-protein together with other soluble substances from the cell and the sheaths. This slightly turbid solution was brilliantly fluorescent. Its fluorescence was many times brighter than that of the living plant. We believe that the act of grinding in distilled water diluted the cell sap to the point where the phycocyanin-chlorophyll distance became so great that internal conversion was no longer important. The solution was stable at room temperature both in the light and in the dark. Since the absorption bands appeared to be in the same position as in the living material, there is little reason to believe that the chlorophyll-protein and the phycocyanin-protein were not in much the same condition as in the living plant.

Dr. C. B. van Niel, by adding ammonium sulfate until the chlorophyll-protein had precipitated, then centrifuging and diluting, was able to separate the chlorophyll-protein from the phycocyanin-protein. While we were no longer sure that the pigment-protein complexes were not changed in some subtle way, we had at least for the determination of the chlorophyll cross-section to work with the separated components.

The phycocyanin-protein solution was examined with a spectroscope; it was found that the fluorescent light is distributed in a band between 6200 and 6550 Å. A determination of the fluorescent yield was made and found to be about 20 per cent. Both of these measurements were the same when made on the original mixture of chlorophyll-protein–phycocyanin-protein. The chlorophyll-protein fraction was divided into two parts. To one methyl alcohol was added to extract the chlorophyll off the protein, then ether and ammonium sulfate solution were added so that the chlorophyll went into the ether fraction. This was separated from the water phase, diluted with methyl alcohol, and the chlorophyll determined as before. This determination gave 8.2×10^{15} chlorophyll molecules per cc. for the chlorophyll-protein solution. The second part was put in the Colman spectrophotometer and the transmission measured as a function of wave length. We then calculated the cross-section per chlorophyll molecule from the equation

$$I = I_0 e^{-\sigma c x}$$

where σ = cross-section in cm^2. per molecule
c = concentration of chlorophyll = 8.2×10^{15} molecules/cc.
x = 1.30 cm. = thickness of our absorption cell.

The results are given in Table I.

We see that over the region 6200 to 6550 Å (within which the fluorescent light

is distributed) the cross-section for the chlorophyll changes from 0.55 to 1.15 $\times 10^{-16}$ cm.^{2}. Since we do not know the distribution of the intensity within the band we cannot integrate and take an average value. We will use the value 0.7×10^{-16} cm.^{2} in our calculations and we will use 6380 Å as the wave length of the fluorescent light. In each case we must remember the possible variation.

In order to estimate F, the fluorescent yield for phycocyanin in the living cell, we illuminated a suspension of photosynthesizing cells in a square glass vessel 1 cm. on each side; the suspension was also 1 cm. deep. The light was admitted from the side and was limited by filters to the wave lengths between 6000 and 6700 Å. Alongside of the vessel containing the cells was placed a block of magnesium carbonate at an angle of 45° with respect to both the original light beam and the direction of view. By looking down from the top it was pos-

TABLE I

Cross-Section of Chlorophyll (in the Chlorophyll-Protein Complex) as a Function of Wave Length

λ	$\frac{I_0}{I}$	cm.^{2}
Å		
6000	1.72	0.51×10^{-16}
6100	1.76	0.53
6200	1.80	0.55
6300	1.82	0.56
6400	2.10	0.70
6500	2.70	0.93
6600	4.56	1.42

sible by placing neutral filters between the light source and the magnesium carbonate block to adjust its brightness to match that of the fluorescing cells. The balance point was found to be given by the combination of 5 to 10 to 50 per cent neutral filters. Thus the brightness of the magnesium-carbonate block will be given by

$$k \times 0.05 \times 0.10 \times 0.50 \times 0.7 = k \times 1.8 \times 10^{-3}$$

where k is a constant depending upon the source and 0.7 is the cos 45°.

The suspensions that we used have been measured in the spectrophotometer and shown to have a transmission of about 40 per cent for a depth of 1.3 cm. for these wave lengths. If we assume that the light absorption by the suspension follows the equation

$$I = I_0 e^{-\epsilon x}$$

then ϵ is equal to 0.7.

We can now write down an expression for the brightness of the suspension looked at from above. It will be:

$$k \times F \times \frac{1}{2} \times 0.7 \times \frac{5}{7} \times \frac{1}{2} = kF\ 0.13$$

where

F is the fraction of the absorbed light that is fluoresced by the phycocyanin; that is, the efficiency of the fluorescence. The factor $\frac{1}{2}$ comes from the fact that the cells can radiate into the solid angle $4\ \pi$ while the magnesium carbonate gives its light into the angle $2\ \pi$. The next factor 0.7 is just the ϵ that we have mentioned. The $\frac{5}{7}$ factor comes from the integral

$$\int_0^1 \epsilon^{-0.7x}\, dx$$

and gives the shading of the suspension for the fluorescent light. The final factor of $\frac{1}{2}$ comes as an estimate from Emerson and Lewis's curve that phycocyanin will absorb about half of all the light in the 6000 to 6700 region.

By setting the two expressions for brightness equal we have

$$kF\ 0.13 = k\ 1.8 \times 10^{-3}$$

or

$$F = \frac{1.8 \times 10^{-3}}{0.13} = 1.5 \times 10^{-2} = 1.5 \text{ per cent}$$

DISCUSSION

In the first section we have shown that the results of Emerson and Lewis require that, in *Chroococcus*, a light quantum absorbed by phycocyanin has a chance to do photosynthesis that is 90 per cent as large as if it had been absorbed by chlorophyll.

The next section shows that because there are in the living cell many chlorophyll molecules inside of the wave zone, that is closer than several wave lengths from the phycocyanin molecule, we have a transfer of energy from phycocyanin to chlorophyll analogous to internal conversion (see Oppenheimer, 1941) and that this transfer will have a rate ρ times faster than the rate of emission of fluorescent light, by phycocyanin in the cell, where

$$\rho = \frac{\sigma n \lambda^4}{d^3}.$$

The final section gave experimental determinations of σ, n, and λ from which we will now calculate the possible value of the internal conversion coefficient ρ.

The quantity λ that appears in the equation is the average wave length of

the fluorescent light divided by $2\pi\mu$ where μ is the index of refraction for the cell sap.

$$\lambda = \frac{6380\ \text{Å}}{2\pi\mu} = \frac{6380 \times 10^{-8}}{2\pi\ 1.33} = 7.6 \times 10^{-6}\ \text{cm.}$$

Due to the gelatinous sheaths we could not obtain the chlorophyll concentration inside the cell by simply determining chlorophyll in a known volume of packed cells, but we had to use the method of determining the average chlorophyll content per cell and then divide by an estimated volume for the cell determined by microscopic examination. The vertical columns in Table II correspond to the limits in the value of the chlorophyll concentration determined in this way.

The quantity d, which is essentially the distance beyond which a phycocyanin molecule sees a uniform distribution of chlorophyll, is some unknown function of the chlorophyll concentration n. The maximum value of d, and thus a minimum value for ρ, would seem to be that found by considering the phycocyanin molecule to be at center of the small cube formed by 8 chlorophyll molecules when all the chlorophyll is arranged in a regular cubic array.

$$d\ \text{max.} = \frac{\sqrt{3}}{2} n^{-\frac{1}{3}}$$

We feel that the most likely value of d is the radii of the little spheres given by

$$\frac{4}{3}\pi d^3 n = 1$$

The value of d, calculated from these two expressions, and using our estimates of the chlorophyll concentration, varies between 7 and 40 Å. These are to be compared with the value of 75 Å given by Förster (1946) as the order of magnitude of the distance between two similar molecules (fluorescing at 6000 Å) at which the resonance transfer of energy will become important.

In Table II we give the internal conversion coefficients in the first row for the maximum value of d and in the second row for the most likely value.

While the variation 36–715 is somewhat embarrassing, the table does show that the internal conversion coefficient is a number of the order of magnitude of 100 and thus that this energy transfer is a most likely explanation of the Emerson and Lewis results.

We measured the efficiency of fluorescence in the living plant, as described in the experimental section, and found that $1\frac{1}{2}$ per cent of the energy absorbed by phycocyanin was readmitted as visual light. The method of making the measurement was not very elegant, and certainly these criticisms can be made: (1) the light on the two surfaces whose brightness must be compared is not of exactly the same color, 6200–6550 Å in one case, 6000–6700 Å in the other; (2) no correction was made for the scattering of the exciting light by the cells; (3)

the estimation of the fraction of the light absorbed by phycocyanin may be wrong. Nevertheless, the measurement cannot be seriously in error as the following argument will show. The fluorescence of chlorophyll in plants like *Chlorella* has been measured a number of times and found to be about 0.15 per cent. Fluorescence in *Chlorella* can only be seen if we take considerable trouble, while the fluorescence in *Chroococcus* is one of the most striking characteristics of the plant material. It can easily be seen in the ordinary lighted laboratory with a small flashlight. Thus it certainly is many times larger than 0.15 per

TABLE II

Internal Conversion Coefficient, Phycocyanin to Chlorophyll

The distance d phycocyanin to chlorophyll	Chlorophyll concentration inside the cells	
	Lower limit 1×10^{19} $\frac{\text{molecules}}{\text{cc.}}$	Upper limit 2.7×10^{19} $\frac{\text{molecules}}{\text{cc.}}$
Upper limit $d = \frac{\sqrt{3}}{2} n^{-\frac{1}{3}}$	36	262
Probable value $\frac{1}{d^3} = \frac{4\pi}{3} n$	98	715

TABLE III

The Probable Disposition of the Energy Absorbed by Phycocyanin in Chroococcus

Emitted as fluorescent light	1 per cent	2 per cent
Degraded to heat	4 " "	8 " "
Transferred to chlorophyll by internal conversion and thus available for photosynthesis	95 " "	90 " "
	100 per cent	100 per cent
Internal conversion coefficient needed	95	45

cent. On the other hand, the cell material is much less fluorescent than is the phycocyanin-protein solution that has an efficiency of 20 per cent. Taking the fluorescent yield of the living plants to be between 1 and 2 per cent, and taking the fact that the fluorescent efficiency of the phycocyanin-protein solutions is 20 per cent to mean that the process whereby energy is degraded to heat is four times as fast as fluorescence, we can construct a table showing how the energy absorbed by phycocyanin in *Chroococcus* is disposed of (Table III).

If the ideas outlined in this paper are accepted then there follow several consequences that we will mention briefly.

Since the chlorophyll mechanism does the photosynthesis, whether the en-

ergy is absorbed by the chlorophyll or by the phycocyanin, it follows that the effect of inhibitors, poisons, narcotics, temperature, light intensity, etc., will be the same on the two kinds of photosynthesis. We have studied the effect of ultraviolet light (Arnold, 1933) on both *Chroococcus* and *Chlorella* with the idea in mind that if the phycocyanin photosynthesis mechanism is separate from the chlorophyll mechanism, then the log survival curves for photosynthesis in *Chroococcus* should break into two sections with different slopes just as do the activity curves for a sample composed of two different β-ray–emitting elements. The results of the experiment give no reason to believe that phycocyanin has its own photosynthetic apparatus.

The fluorescence of the chlorophyll should be the same whether the exciting light is absorbed by chlorophyll or by phycocyanin. The demonstration of this point would constitute a proof that energy was transferred from phycocyanin to chlorophyll. We attempted to carry out the experiment but the work was interrupted by the war before any results were obtained. Since then it has been shown by Dutton, Manning, and Duggar, (1943) and by Wassink and Kersten (1946), that in the case of the diatoms the fluorescence of chlorophyll does take place when the exciting light is absorbed by fucoxanthin. We can make no calculations about internal conversion in the diatoms since we do not know whether or not fucoxanthin is fluorescent.

In the case of the red algae and phycoerythrin it would seem that internal conversion might be an important energy-transferring system. While it may be true that the cross-section of chlorophyll for the fluorescent light of phycoerythrin is considerably less than it is for 6200–6550 Å, the fact that both chlorophyll and phycoerythrin are concentrated in chloroplasts will increase the value of n and decrease the value of d so that we would expect to again obtain a high value for the internal conversion coefficient. Dr. Blinks pointed out to us that the fact that when red algae were damaged in such a manner that the phycoerythrin leaches out of the chloroplast (thus greatly increasing d and decreasing internal conversion) the fluorescence of the plants becomes a great many times brighter, can be used as an argument that internal conversion does take place. However, since then, Blinks (1947) has reported that he found red algae in which photosynthesis took place when light was absorbed in the blue absorption band of chlorophyll and when it was absorbed by phycoerythrin, but not when light was absorbed by the red absorption band of chlorophyll. What this means we do not know. Apparently much further experimental work needs to be done before we can understand what is happening in the case of the red algae.

We expect that energy will be transferred from phycocyanin to phycocyanin and from chlorophyll to chlorophyll by this same internal conversion process. A rough estimate of the internal conversion coefficients gives about 1.5 for the phycocyanin and perhaps 4 for the chlorophyll. One is tempted to speculate on

the possibility that we have here a method for the transfer of energy through the chloroplast.

SUMMARY

1. In *Chroococcus* a quantum of light absorbed by phycocyanin has 90 per cent the chance of doing photosynthesis that a quantum absorbed by chlorophyll has.

2. By a process analogous to internal conversion in radioactivity (but with the linear dimensions and the wave length 10^4 times larger) there will be transferred from phycocyanin to chlorophyll $\frac{\sigma n \lambda^4}{d^3}$ (a number of the order of 100) quanta for every one emitted as fluorescent light by the phycocyanin in the *Chroococcus* cell.

3. The yield of fluorescent light in *Chroococcus* is between 1 and 2 per cent.

4. The transfer of energy by internal conversion can account for the photosynthesis by phycocyanin observed by Emerson and Lewis.

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