THE EFFECT OF ULTRAVIOLET LIGHT ON PHOTOSYNTHESIS

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I

In studying the killing of small organisms by x-rays, α-rays, and ultraviolet light, a number of investigators (Crowther, etc. (1)) have found that the results could be most simply explained by the assumption that one quantum or one α-particle caused death by hitting a cell. These results are of interest for two reasons. First, quantum mechanisms may possibly exist in living material. Second, the method of studying small structures by their interaction with photons is not subject to the limitations imposed on the microscopic method by the wave nature of light. (An example of this is DuMond's recent investigation—by the Compton effect—upon the magnitude and distribution of velocities of electrons inside the atoms of solid metals (DuMond (2)).)

This paper has to do with the effect of the ultraviolet radiation (Hg 2537 Å) on the green alga Chlorella pyrenoidosa. However, death will not be used as an end-point because it might result from a number of different causes and is difficult to define and to test for. Instead, attention will be concentrated on some function of the cell—respiration, fermentation, or photosynthesis—on the assumption that the mechanism of that function is more uniform in its sensitivity to the radiation than are the cells themselves. The effect of the radiation on this function will be studied manometrically by the method described by Warburg (3) and Emerson (4). This method allows the use of a far larger number of individuals than can be used when counts must be made. One experiment involves 150 million cells, thus reducing the rôle of statistical variation to a minimum.
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II

The device for determining the number of quanta involved is as follows:

One Quantum Hit

If we have \( N \) individuals of Type A, one quantum can change an A to a B.

\[ A + h\nu \rightarrow B; \]

the rate of destruction of A is given by

\[ \frac{dN}{dt} = NQP \tag{1} \]

where \( Q \) is the rate of absorption of quanta by one A.

\[ Q = \frac{I\mu V}{h\nu} \]

when

\( I = \) light intensity
\( \mu = \) absorption coefficient
\( V = \) volume of A

and \( P \) is the probability of an absorbed quanta effecting the change A to B. (\( P \) admits the possibility of an absorbed quantum not making a change in A.)

The solution of equation (1) is—

\[ N = N_o e^{-QPt}, \tag{II} \]

\( N_o = \) number of A present before irradiation;
\( t = \) time of irradiation.

Equation (II) gives

\[ \ln \left( \frac{N}{N_o} \right) = -QPt \tag{III} \]

Plotting \( \ln \left( \frac{N}{N_o} \right) \) (survival ratio) against time gives the graph shown in Fig. 1. The value of the slope will be \(-QP\).
If it takes two quanta to effect the change of A to B, we obtain:

$$A + h\nu \rightarrow A'$$

where $A'$ cannot be distinguished by the experiment from $A$.

$$A' + h\nu \rightarrow B$$

$B$ = the "killed" or inactive form of $A$.
$Q$ = the rate at which quanta are absorbed by one $A$ or $A'$.
$P$ = the probability of a hit being effective. (It should be stated that $P$ might be different for $A$ and for $A'$.)

$N_0$ = number of units of $A$ before irradiating.
$N$ = number of units of $(A + A')$ at any time.
$t$ = time of irradiation.
$S$ = number of units of $A$ at any time.
From equation (II) we have

\[ S = N_0 e^{-QPt}. \]

\[- \frac{dN}{dt} \] will be proportional to the number of A's present, that is, to \((N - S)\):

\[- \frac{dN}{dt} = (N - S) Q P = Q P (N - N_0 e^{-QPt}). \]

\[ N_0 \]

The solution is

\[ N = e^{-QPt} \left(QPN_0 + C\right). \]  

\(N = N_0\) when \(t = 0\), so that the constant \(C = N_0\) and

\[ \frac{N}{N_0} = e^{-QPt} (1 + QPt); \]
taking the log of each side we have

\[ \ln \left( \frac{N}{N_0} \right) = -QPt + \ln (1 + QPt). \]  

Plotting \( \ln \) (survival ratio) against time, we have the curve given in Fig. 2. A comparison of Figs. 1 and 2 shows the possibility of deter-

mining the number of quanta involved in the change from the shape of the curve for \( \ln \) (survival ratio) against time. As the number of quanta involved increases, the curves shift progressively to the right (Curie (1)).

III

For the experiments 5 mm.\(^2\) (150 million) of Chlorella pyrenoidosa cells were suspended in 1.5 cc. of Warburg's carbonate buffer (Mixture IX), (Warburg (3)), in a small quartz Warburg vessel. Photosyn-
thesis and respiration were measured as a function of the time of irradiation. The cells were rayed in the vessel with the light from a quartz mercury tube. It is possible by electrodeless discharge in mercury vapor induced by a radio frequency coil (λ = 3M.) to obtain light from 90 to 96 per cent monochromatic for the 2537 Å line.

When the ln (survival ratio) for respiration and for photosynthesis are plotted against the time of irradiation we obtain the kind of result shown in Fig. 3.

\( P \), the probability of a hit being effective, may be determined from the following experiment. 5 mm.\(^2\) of cells were used as before. The energy output of the ultraviolet tube was measured by Dr. F. L. Gates and found to be 37.4 ergs per mm.\(^2\) per second at the point where the quartz vessel was placed. The area exposed was 160 mm.\(^2\). Measurements with a Weston cell (quartz window) showed that 52 per cent of the incident light was absorbed by the \textit{Chlorella} suspension. This means that the cells absorbed 3.1 \times 10^8 ergs per second. The energy of one quantum of 2537 Å wave length is 7.7 \times 10^{-12} ergs. By dividing this number into the energy absorbed we obtain 4 \times 10^{14} as the number of quanta absorbed per second.

The numerical value of \( P \) depends upon what element we assume to be destroyed by the ultraviolet light. Table II gives the calculations for three possible assumptions: first, individual \textit{Chlorella} cells; second, photosynthetic units; third, chlorophyll molecules.

According to hemocytometer counts there are 30 million \textit{Chlorella} cells per mm.\(^2\) of the cell material.
Defining the number of photosynthetic units as the number of carbon dioxide molecules reduced per flash when the flashes are so far apart that they do not interfere with one another, and when the flashes are bright enough to produce light saturation—then the number of photosynthetic units is

\[
\frac{13.2 \times 0.185 \times 6.06 \times 10^{11}}{5 \times 60 \times 15 \times 1000 \times 22400} = 1.46 \times 10^{11}
\]

where

13.2 mm. = the change of the manometer pressure in 5 minutes (determined previous to the irradiation by ultraviolet light).

0.185 = the vessel constant

15 = the number of flashes per second.

### TABLE II

<table>
<thead>
<tr>
<th>Element assumed to be hit by ultraviolet light</th>
<th>( N )</th>
<th>( Q )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorella cell</td>
<td>( 150 \times 10^9 )</td>
<td>( 2.7 \times 10^8 )</td>
<td>( 6.3 \times 10^{-10} )</td>
</tr>
<tr>
<td>Photosynthetic unit</td>
<td>( 1.46 \times 10^{12} )</td>
<td>( 2.7 \times 10 )</td>
<td>( 6.3 \times 10^{-5} )</td>
</tr>
<tr>
<td>Chlorophyll molecule</td>
<td>( 3.6 \times 10^{10} )</td>
<td>( 1.1 \times 10^{-2} )</td>
<td>( 1.5 \times 10^{-1} )</td>
</tr>
</tbody>
</table>

The number of chlorophyll molecules is equal to the number of photosynthetic units multiplied by 2480 (Emerson and Arnold (5)). Although the relative probabilities are correct, the absolute magnitudes are subject to an error perhaps as large as 500 per cent. It has not been possible as yet to grow two cultures giving identical slopes. Furthermore, the light intensity has not been corrected for reflection, or for absorption by different parts of the cell. A new absorption cell is now being made with which the absorbed energy can be determined with greater precision.

The value \( 1.5 \times 10^{-1} \) for \( P \) suggests at once that it is the chlorophyll molecule which is affected by ultraviolet light. To test this assumption arrangements were made with Professor J. B. Conant and Dr.
Dietz to examine the chlorophyll chemically. A culture of *Chlorella pyrenoidosa* was irradiated with ultraviolet light until manometric tests showed that the photosynthesis had been reduced to less than 10 per cent of its original rate. The culture was then given to Dr. Dietz who made extracts within less than 1 hour from the time of irradiation. Previous measurements had shown that the damage to photosynthesis by ultraviolet light lasts for at least 7 hours. The following is Dr. Dietz’ report:

“The suspension of *Chlorella* was centrifuged, washed once with distilled water and the chlorophyll was extracted by grinding with sand in the presence of acetone. Ether was added to the acetone solution and the acetone removed by washing carefully with water.

Suitable tests showed that the chlorophyll was unchanged chemically. A prolonged yellow phase color was obtained on shaking the ether solution with methyl alcoholic potassium hydroxide, hence no allomerization had taken place. Neither 0.01 N potassium hydroxide nor 22 per cent hydrochloric acid extracted any of the pigment, hence the phytol group had not been removed. A hot quick saponification carried out according to the Willstätter procedure followed by methylation and acid fractionation indicated that chlorin e and rhodin g esters were the sole products and were formed in the normal 3 to 1 ratio. This showed that no oxidation of the chlorophyll or alteration of the ratio of the a and b components had taken place.”

**IV**

**CONCLUSIONS**

The fact that the chlorophyll appears to remain unchanged chemically allows two conclusions,—either that there is a change so subtle that it escapes detection, or that the ultraviolet light destroys a substance other than chlorophyll. If the first proves true, then a mechanism like that suggested by Conant, (Conant, Dietz, and Kamerling (6)) may be used to explain the high ratio between chlorophyll content and photosynthesis per flash (Emerson and Arnold (5)). If the second is the correct conclusion, then the hypothetical substance must be proportional to, and a very small fraction of, the chlorophyll content of the cell. The probability calculated for the photosynthetic unit fits this conclusion—because most of the absorbed quanta would be taken up by the chlorophyll which is present in a much higher concentration than is the hypothetical substance. However, Warburg’s high light
efficiency for *Chlorella* is difficult to understand from this point of view. We would expect the chlorophyll to act as an internal screen.

It is probable that when the mechanism of the photosynthesis of green plants is finally described, it will be found that the study of the quantum relationships of both the visible and ultraviolet light have played an important part.

The writer is much indebted to Dr. E. M. Dietz, Professor J. B. Conant, Professor W. J. Crozier, and Dr. F. L. Gates for help and advice.

**SUMMARY**

1. An unidentified unit in the mechanism of the photosynthesis of *Chlorella pyrenoidosa* is rendered inactive by the absorption of one quantum of ultraviolet light (2537 Å wave length).
2. The same irradiation has no effect on the normal respiration of *Chlorella pyrenoidosa*. Experiments have not yet been made on the respiration inhibitable by HCN.
3. No chemical change was detected in the chlorophyll extracted from irradiated cells.

**CITATIONS**