PHYSIOLOGICAL EFFECTS OF RADIANT ENERGY

by

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PHYSIOLOGICAL EFFECTS OF RADIANT ENERGY

INTRODUCTION.

Since the very earliest times light has been worshipped as the giver of health and as the enemy of darkness and of death, but it is only in comparatively recent years that scientific enquiry has been carried on as to its actual biological and physiological effects. The amount of literature on the subject is immense and varied, so that it is impossible to give a comprehensive account of any but the results most pertinent to the work described in this discussion.

For the past four years work on the effect of Radiant Energy on Protoplasma has been carried on under the direction of Dr. Hutchinson and in order to understand the reasons leading up to the investigation of enzyme action outlined in the latter portion of this paper, it is necessary to describe the preliminary work.

It was therefore thought advisable to divide this paper into two parts, A and B; A to consist of a description of the work on Protoplasma and B to consist of a description of the effect of Radiant Energy on enzyme action in particular.
ACKNOWLEDGMENTS

Grateful acknowledgment is made to Dr. A. H. Hutchinson, under whose direction this work has been done, for his unfailing help and encouragement.

The author is also indebted to the National Research Council of Canada for the use of light sources and instruments in their possession.

..........................
PART A.

THE EFFECT OF RADIANT ENERGY ON PROTOPLASM.

INTRODUCTION.

Man's control of the effects of light and more especially ultra violet light is at present incomplete and although the effects may differ with wave length and intensity and with the subject irradiated little is known as to the why or the wherefore of the results obtained.

It was thought that a comparison between the effect of Radiant Energy and more particularly Monochromatic Light on animal and plant protoplasm might help towards the understanding of these phenomena and towards their more helpful utilization.

REVIEW OF LITERATURE.

1. Effect of Light on Animal Protoplasm.

Light can have no effect unless it is absorbed and according to Bovie (3) "rays affect protoplasm at the place where they are absorbed, and observed physiological disturbances are the responses on the part of the organism to its injured protoplasm". In amplification of this statement Bovie and his co-workers (4) have demonstrated the effect of various light rays on Protoplasm. Paramecium caudatum was exposed to ultra violet light in a
fluorite chamber and the organism was rendered extremely sensitive to heat, so much so that it was killed by an amount of heat which would not affect it normally. The Schumann region (5) is a region of general absorption for nearly all substances and has an immediate effect on infusoria, causing marked stimulation followed by cytology and with sufficient exposure, death. Packard (25) finds that Paramecium is susceptible to Radium radiations and that the susceptibility to these radiations varies with the permeability of the surface layer of the cell. He suggests that the increased permeability is the cause of the acceleration in the division rate observed. Hutchinson and Ashton (18) found that moderate increase in rate of plasmolysis accompanied increase in cell division in the same organism and that an excessive increase in rate of plasmolysis accompanied decrease in growth rate, but whether this change in plasmolytic rate was due to a changed permeability or to the change in the osmotic content of the cell has not been determined.

According to Hughes and Bovie (16) cytology in Paramecium can be caused by exposure to ultra violet light, an exposure sufficient to cause cytology inhibiting cell division. With short exposures the inhibition is transitory and is followed by an increase in cell division over that of the control.
2. Light responses - Animal.

Work on multicellular organisms shows that radiant energy has a profound effect on growth and development. Loeb (23) found that unfertilized eggs of Arbacia, when treated with rays from a quartz mercury lamp formed a fertilization membrane and in some cases developed into larvae, though not proceeding beyond the gastrula stage. Higgins and Sheard (15) found that ultra violet light effected the development of the eggs of Rana pipiens and that the regions of highest metabolic activity were most responsive to radiation. X-rays have an even more far reaching effect. Mullar and Weinstein (37) obtained mutations, producing visible and lethal effects as well as genetic modifications of frequency of crossing over in Drosophila exposed to these rays and attachment between genes of different chromosomes, pointing to a profound effect on the reproductive cells and the chromosomes themselves.

Reaction to specific wave lengths has also been recorded. Higgins and Sheard (14) found that in the absence of ultra violet light, as when blue or amber screens were used, hyperplasia of the parathyroid glands of chickens resulted, and that the best development took place in direct sunlight. Abbot (1) states that Formica dakotensis specularis responded to light of the same intensity in the following order, yellow, white, red. Visscher and Luce (36) found that 5300 Å-5450 Å were most effective in causing reaction on the larvae of barnacles. There is a decrease in effectiveness to 4200 Å
and a distinct stimulating effect in the ultra violet.

For the cure of rickets in animals ultra violet light of various wave lengths is efficacious. G. E. Maugham (24) showed that the wave lengths between 3132 A° and 2650 A° are most effective in this respect. The curative effect of wave lengths shorter than 2896 A° seemed to be small and that of wave length 3132 A° itself to be nil. The result of careful analysis showed that 2968 A° was the most effective for the cure of rickets and that 3024 A° was perhaps a fourth as potent.

3. Light responses—Plants.

Priestley (29) has performed experiments on the growth of seedlings that show that brief exposure to light has very marked effects. A light exposure of one to two minutes per day to a relatively weak artificial light will remove effectively the most characteristic morphological features of etiolation, while the expanded leaf will fail to produce any chlorophyll. Higgins and Sheard (14) found that the lesser wave lengths used, 3200 A°-2700 A°, stimulated and accelerated the time of germination while greater wave lengths inhibited germination, growth being accelerated by irradiation in the near ultra-violet, 4000A°-3000 A°; 2900 A° and below being definitely lethal.

Notable results have been obtained in plants by Goodspeed and Olsen (11). Tobacco in full flower was exposed
to X-rays. Plants grown from seeds irradiated at this early stage included many variations. A cytological investigation showed that chromosome disturbances had occurred and that these modifications were in all probability primary to the external variations in form.

Popp (28) removed definite regions of the spectrum in the blue-violet and noted the effect on plants. Eliminating all lines below 5290 Å resulted in plants having an etiolated appearance. Eliminating below 4720 Å produced the same effect, but after elimination of all ultra violet very little difference was seen. From this result it may be inferred that ultra violet rays are not indispensable but that the blue violet end of the spectrum is necessary for normal vigorous growth.
EXPERIMENTAL WORK.

Part 1. Effect of monochromatic light on Paramoecium

The first part of the experimental work was to ascertain the effect of Monochromatic Light on animal protoplasm, Paramoecium caudatum being the organism used.

The apparatus used throughout all the following work was a Cooper Hewitt "Lab-arc" for the source of light. It was operated on an alternating current of 110 volts with a resistance of 12 ohms in series and functioned under these conditions as a low pressure mercury arc.

A Monochromatic Illuminator of Adam Hilger, Ltd., fitted with quartz prism and lenses and reading directly to wave lengths was used to resolve the light. The instrument was standardised by readings on the sodium D line.

Paramoecia were placed in a quartz cell which fitted into the slot of the monochromatic illuminator, the control being kept in a similar cell in the dark.

The effect of various monochromatic lines of the Mercury Arc Spectrum on the rate of division of the organism was ascertained and also the effect on the rate of plasmolysis.

DISCUSSION OF RESULTS.

1. Rate of Growth. (Table 1.)

From the accompanying graph (Plate 1.) it will
Stimulation and Retardation of Growth in Paramoecium

expressed as

number of Paramoecia

greater or less than in the control.
<table>
<thead>
<tr>
<th>Wave Length in A</th>
<th>Number of Paramaecium after 6 hours</th>
<th>Number of Paramaecium after 24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Irradiated</td>
<td>Control</td>
</tr>
<tr>
<td>6152</td>
<td>13.4</td>
<td>12.8</td>
</tr>
<tr>
<td>5819-5769</td>
<td>12.85</td>
<td>12.85</td>
</tr>
<tr>
<td>4968-4916</td>
<td>9.1</td>
<td>13.0</td>
</tr>
<tr>
<td>4359-4348</td>
<td>14.1</td>
<td>13.0</td>
</tr>
<tr>
<td>4078-3984</td>
<td>13.2</td>
<td>13.4</td>
</tr>
<tr>
<td>3821</td>
<td>11.4</td>
<td>11.3</td>
</tr>
<tr>
<td>3663-3656</td>
<td>12.1</td>
<td>11.3</td>
</tr>
<tr>
<td>3562-3542</td>
<td>11.5</td>
<td>10.5</td>
</tr>
<tr>
<td>3132</td>
<td>12.3</td>
<td>12.1</td>
</tr>
<tr>
<td>3022</td>
<td>12.0</td>
<td>13.0</td>
</tr>
<tr>
<td>2967</td>
<td>10.4</td>
<td>11.6</td>
</tr>
<tr>
<td>2804</td>
<td>9.9</td>
<td>11.9</td>
</tr>
<tr>
<td>2700</td>
<td>10.4</td>
<td>12.2</td>
</tr>
<tr>
<td>2535</td>
<td>10.0</td>
<td>14.2</td>
</tr>
<tr>
<td>2054</td>
<td>9.9</td>
<td>13.8</td>
</tr>
</tbody>
</table>
be seen that there are two regions which definitely and consistently inhibit the growth rate of Paramoecium; that region around 4960 Å, the indigo blue end of the spectrum, and that beyond 3132 Å, the ultra violet and far ultra violet portion. There also seem to be three regions of stimulation, the yellow orange, 5916 Å and the violet indigo, 4359 Å, and a slight stimulatory effect at the beginning of the ultra violet 3821 Å-3342 Å, but at no time does the magnitude of stimulation approach that of the most extreme results of retardation.

Wave length and not intensity is the determining factor, for the greatest results are obtained with many of the lines of least intensity (ex. 2054 Å, 4960 Å). In cases of slight and transitory inhibition of growth rate, a period of acceleration follows (5819 Å, 3821 Å and 3132 Å), sufficient to result in an actual increase in the number of irradiated individuals as compared to non-irradiated control; should the period of inhibition be prolonged the organism does not recover its normal growth rate and in extreme cases total inhibition results (3022 Å onwards).

These findings correspond in general with the results of Bovie and Hughes (16) for ray 2800 Å, where cytolysis of Paramoecium was caused by prolonged irradiation and exposure insufficient to produce cytolysis caused inhibition of cell division followed by stimulation. No effect with slight
11.

Irradiation may be due to the inability of the rays to penetrate and effect the nucleus.

2. Effect on rate of Plasmolysis. (Table 2.)

The effects of monochromatic light on the rate of plasmolysis in Paramococmum, using varying concentrations of NaCl, show that there are again two or perhaps three regions of greatest effect. (Plates 2 and 3.)

In no case was a decrease in rate noticed, even with the lines causing most effect either in stimulation or inhibition of growth; all wave lengths seemingly having some stimulatory effect on plasmolytic rate. After six hours irradiation the results are a little erratic, those lines around 3650A°-3082 A° being contradictory, but even after this short time three regions causing increase in rate can be noted. After twenty-four hours the regions 4960 A°, 4078 A°, and 3022 A° down to the far ultra violet cause the greatest increase in rate.

These results when compared with the effect on the rate of growth of Paramococmum show a marked similarity, (see plates 2 and 3.) the regions causing most inhibition in growth corresponding to those causing greatest increase in rate of plasmolysis. Those regions causing little effect on growth, or stimulating slightly, correspond with those causing the least increase in the rate of plasmolysis.
12.

**TABLE 2/**

Effect of various wave-lengths of light on the rate of plasmolysis of *Paramaecium caudatum*.

Increase in rate of plasmolysis expressed as percentage increase over control rate.

<table>
<thead>
<tr>
<th>Wave Length in A°</th>
<th>Sodium Chloride Solutions</th>
<th>0.7 Molar</th>
<th>0.3 Molar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6 hours</td>
<td>24 hours</td>
</tr>
<tr>
<td>6152</td>
<td></td>
<td>30</td>
<td>29</td>
</tr>
<tr>
<td>5819-5769</td>
<td></td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>4960-4916</td>
<td></td>
<td>41</td>
<td>39</td>
</tr>
<tr>
<td>4359-4348</td>
<td></td>
<td>20</td>
<td>34</td>
</tr>
<tr>
<td>4079-3984</td>
<td></td>
<td>39</td>
<td>39</td>
</tr>
<tr>
<td>3821</td>
<td></td>
<td>26</td>
<td>40</td>
</tr>
<tr>
<td>3663-3666</td>
<td></td>
<td>28</td>
<td>27</td>
</tr>
<tr>
<td>3358-3342</td>
<td></td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>3132</td>
<td></td>
<td>26</td>
<td>21</td>
</tr>
<tr>
<td>3022</td>
<td></td>
<td>44</td>
<td>18</td>
</tr>
<tr>
<td>2967</td>
<td></td>
<td>34</td>
<td>36</td>
</tr>
<tr>
<td>2804</td>
<td></td>
<td>40</td>
<td>51</td>
</tr>
<tr>
<td>2054</td>
<td></td>
<td>51</td>
<td>51</td>
</tr>
</tbody>
</table>
Comparison of the effect on growth of Paramecium and on plasmolysis in sodium chloride solutions.

Plasmolysis expressed as percent increase in rate.
Comparison of the effect on growth of Paramoecium and on plasmolysis in sodium chloride solutions.

Plasmolysis expressed as percent increase in rate.
Thus it would seem that there is a correlation between division rate and plasmolytic rate in Paramoecium.

Packard (26) working on the same organism found that the permeability of the cells exposed to light was greater than the controls kept in darkness. The change in permeability was also demonstrated in cells exposed to monochromatic red light, becoming greater as the wavelength shortened and reaching a maximum in the near ultra-violet.

A change in the rate of plasmolysis may be caused by various factors. Blackman and Paine (2) record that light has a marked effect on the permeability of the cells of Mimosa pudica to electrolytes, probably due to the "disappearance or inactivation of the osmotic substances of the cell". On the other hand light being absorbed by the cell membrane, it is probable that the membrane itself changes its permeability, perhaps due to the action of light on the lipoid substances which are believed to be one of its constituents.

It can be definitely stated, however, that conditions accompanying moderate increase in rate of plasmolysis also accompany increase in rate of cell division and that conditions accompanying extreme increase in rate of plasmolysis result in decrease in growth.
Experimental Work.


The experiments to be described deal with the effect of radiant energy on 1) growth 2) sporulation, of Colletotrichum homoides, a common cause of ripe rot of tomatoes(19).

Potato dextrose agar was used as the nutrient medium, single plantings being made on the agar and allowed to grow for three days before irradiation.

Experiment 1. Irradiation with full light from a mercury arc lamp.

1. Single spore cultures of three days growth were exposed to the full light from a quartz mercury lamp at a distance of 12 cm. from the source, part of the culture being protected by a half circle of black cardboard. The line of separation was evident within a few minutes of irradiation, caused by the collapse of the aerial mycelium in the exposed part; in all cases growth was retarded and sporulation accelerated in the area irradiated. (Plate 4).

2. Single spore cultures of three days growth were irradiated for periods of three minutes to thirty seconds with the light from a quartz mercury lamp. It was found that irradiation for three minutes caused stunting
Effect on sporulation and growth of irradiating half of three-day cultures with the mercury vapour arc spectrum, lower side exposed, upper side normal.

A. Culture, 14 days after 4 min. exposure.
B. Culture, 3 days after 15 min. exposure.
from which the

growth rate.

two minutes of
exposure, but

terminated on the
twenty-fourth or
twenty-fifth
development

to one minute

cratering
development.


eventh day of growth.

B. Irradiation of a suspension of spores

A suspension of spores in sterile water was treated with the
fall illumination from a quartz mercury lamp for the desired

then plated and

the culture did

fifteen seconds of
rate gradually

results that the

but it should be

and that the

or less fatal to it than to the older mycelia.


generated, and that the germination tube was exposed to the

take which proved more fatal to it than to the older mycelia.
from which the culture did not afterwards regain the normal growth rate. (Table 3.) Irradiation of from fifteen seconds to two minutes caused stunting in direct relation to the time of exposure, but from which the culture recovered and ultimately exceeded the control in growth. Direct irradiation of the three day culture results after about twenty-four hours in the formation of numerous acervuli, mostly in clumps, the maximum development of acervuli taking place after thirty seconds to one minute exposure, longer periods of irradiation accelerating development but in smaller quantities. Development of acervuli in the control did not usually take place until the eighth day of growth.

3. Irradiation of a suspension of spores.
A suspension of spores in sterile water was irradiated with the full illumination from a quartz mercury lamp and the spores then plated out on bacto potato dextrose agar.

Irradiation of spores for periods of more than thirty seconds caused an intensive initial stunting from which the culture did not regain normal growth rate. Irradiation for fifteen seconds also caused initial stunting but the growth rate gradually approached normal. It would seem from these results that the effect of irradiation is more drastic in the case of the spores than the growing mycelium, but it should be born in mind that many of the spores irradiated may have germinated, and that the germination tube was exposed to the rays which proved more harmful to it than to the older mycelium
TABLE 3.

Effect of full illumination with the mercury arc lamp upon the growth of Colletotrichum.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Days</th>
<th>4th.</th>
<th>5th.</th>
<th>6th.</th>
<th>7th.</th>
<th>8th.</th>
<th>9th.</th>
<th>10th.</th>
<th>11th.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.4</td>
<td>3.28</td>
<td>4.1</td>
<td>4.8</td>
<td>5.6</td>
<td>6.3</td>
<td>7.1</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>3 minutes</td>
<td>2.1</td>
<td>2.5</td>
<td>-</td>
<td>4.1</td>
<td>5.1</td>
<td>5.6</td>
<td>6.6</td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td>2 minutes</td>
<td>2.15</td>
<td>2.4</td>
<td>-</td>
<td>4.3</td>
<td>5.35</td>
<td>6.1</td>
<td>7.1</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>1 minute</td>
<td>2</td>
<td>2.9</td>
<td>-</td>
<td>4.7</td>
<td>5.6</td>
<td>6.3</td>
<td>7.3</td>
<td>7.9</td>
<td></td>
</tr>
<tr>
<td>30 seconds</td>
<td>2.25</td>
<td>3</td>
<td>-</td>
<td>4.9</td>
<td>5.7</td>
<td>6.5</td>
<td>7.5</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>15 seconds</td>
<td>2.3</td>
<td>3.1</td>
<td>-</td>
<td>4.8</td>
<td>5.7</td>
<td>6.5</td>
<td>7.4</td>
<td>7.9</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 4.

Effect of full illumination with the mercury arc lamp upon Colletotrichum spore suspensions.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Days</th>
<th>4th.</th>
<th>5th.</th>
<th>6th.</th>
<th>7th.</th>
<th>8th.</th>
<th>9th.</th>
<th>10th.</th>
<th>11th.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.4</td>
<td>3.28</td>
<td>4.1</td>
<td>4.8</td>
<td>5.6</td>
<td>6.3</td>
<td>7.1</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>2 minutes</td>
<td>2.0</td>
<td>2.7</td>
<td>-</td>
<td>4.0</td>
<td>5.1</td>
<td>5.9</td>
<td>6.5</td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td>1 minute</td>
<td>1.95</td>
<td>2.7</td>
<td>-</td>
<td>4.4</td>
<td>5.1</td>
<td>5.9</td>
<td>6.6</td>
<td>7.4</td>
<td></td>
</tr>
<tr>
<td>30 seconds</td>
<td>2.1</td>
<td>2.9</td>
<td>-</td>
<td>4.6</td>
<td>5.2</td>
<td>6.1</td>
<td>6.7</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>15 seconds</td>
<td>2.1</td>
<td>3.1</td>
<td>-</td>
<td>4.7</td>
<td>5.5</td>
<td>6.2</td>
<td>7.0</td>
<td>7.8</td>
<td></td>
</tr>
</tbody>
</table>
of the three day cultures.

In all cases development of *acervuli* was hastened, being noted in the irradiated culture several days before their development in the control. Maximum development seemed to take place in cultures irradiated for not more than thirty seconds.

**Experiment 2. Irradiation of spores in suspension by monochromatic light.**

A suspension of spores in sterile water was placed in a quartz container in the slit of the monochromatic illuminator and was irradiated with various monochromatic lines of the mercury arc spectrum.

The effect of irradiation of the spores by specific wave lengths was seemingly confusing until divided into four classes. (Table 5.)

**Class 1. Stimulation continuous.**

5819 Å, 4078 Å, 3656 Å and 3342 Å cause stimulation of growth continuing throughout the eleven day period, except in the case of 3342 Å where the maximum stimulation is reached at the fourth day.

**Class 2. Retardation followed by stimulation.**

Irradiation by lines in this class results in a primary retardation of small degree followed by stimulation which is generally in inverse proportion to the initial retardation. This effect is caused by wave lengths
### TABLE 5.

Specific effects of monochromatic light upon the growth of *Colletotrichum* spore suspensions.

<table>
<thead>
<tr>
<th>Wave length in A°</th>
<th>Exp. Size of culture in cms.</th>
<th>4th.</th>
<th>5th.</th>
<th>6th.</th>
<th>7th.</th>
<th>8th.</th>
<th>9th.</th>
<th>10th.</th>
<th>11th. Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>2.4</td>
<td>3.28</td>
<td>4.1</td>
<td>4.8</td>
<td>5.6</td>
<td>5.3</td>
<td>7.1</td>
<td>7.8</td>
</tr>
<tr>
<td><strong>Class 1.</strong></td>
<td>Wave lengths causing consistent stimulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5819</td>
<td></td>
<td>1</td>
<td>2.4</td>
<td>3.2</td>
<td>4.0</td>
<td>5.0</td>
<td>5.7</td>
<td>6.3</td>
<td>7.2</td>
</tr>
<tr>
<td>5869</td>
<td></td>
<td>2</td>
<td>2.6</td>
<td>3.5</td>
<td>4.5</td>
<td>5.7</td>
<td>6.4</td>
<td>7.0</td>
<td>7.4</td>
</tr>
<tr>
<td>4078</td>
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<td>2.6</td>
<td>3.2</td>
<td>4.0</td>
<td>4.9</td>
<td>5.6</td>
<td>6.9</td>
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<td>3984</td>
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<td>3.1</td>
<td>4.1</td>
<td>5.2</td>
<td>6.6</td>
<td>7.6</td>
<td>8.2</td>
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<td>3656</td>
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<td>3.6</td>
<td>4.3</td>
<td>4.7</td>
<td>5.6</td>
<td>6.3</td>
<td>7.1</td>
</tr>
<tr>
<td>3650</td>
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<td>2</td>
<td>2.8</td>
<td>3.5</td>
<td>4.2</td>
<td>5.3</td>
<td>6.0</td>
<td>7.3</td>
<td>7.8</td>
</tr>
<tr>
<td>3342</td>
<td></td>
<td>11</td>
<td>2.9</td>
<td>3.4</td>
<td>3.9</td>
<td>4.8</td>
<td>5.8</td>
<td>6.3</td>
<td>7.1</td>
</tr>
</tbody>
</table>

| **Class 2.**      | Wave lengths causing retardation followed by stimulation. |      |      |      |      |      |      |       |            |
| 6162              |                               | 1    | 2.2  | 3.1  | 4.1  | 4.8  | 5.6  | 6.3   | 7.1        |
| 6162              |                               | 2    | 2.3  | 3.0  | 4.1  | 5.0  | 5.8  | 7.2   | 8.0        |
| 3821              |                               | 1    | 2.3  | 3.1  | 4.5  | 4.9  | 5.4  | 5.8   | 7.0        |
| 3821              |                               | 2    | 2.3  | 3.1  | 4.2  | 5.0  | 6.0  | 6.8   | 7.6        |
| 3132              |                               | 1    | 2.1  | 2.8  | 4.0  | 4.9  | 5.9  | 6.7   | 7.5        |
| 3128              |                               | 2    | 2.3  | 3.2  | 3.7  | 4.8  | 6.1  | 6.8   | 7.6        |
| 2967              |                               | 1    | 2.2  | 3.1  | 3.9  | 4.8  | 5.7  | 6.7   | 7.4        |
| 2700              |                               | 2    | 1.9  | 2.8  | 4.0  | 5.0  | 5.5  | 6.6   | 7.4        |

| **Class 3.**      | Wave lengths causing complete retardation. |      |      |      |      |      |      |       |            |
| 4960              |                               | 1    | 1.9  | 2.9  | 3.8  | 4.6  | 5.6  | 6.3   | 7.0        |
| 4916              |                               | 2    | 1.9  | 2.7  | 3.6  | 4.8  | 5.5  | 6.1   | 6.9        |
| 3022              |                               | 1    | 1.5  | 2.1  | 3.6  | 4.2  | 5.2  | 5.5   | 6.0        |
| 3022              |                               | 2    | 1.4  | 2.1  | 2.8  | 3.5  | 4.2  | 5.7   | 6.3        |
| 2804              |                               | 1    | 1.6  | 2.2  | 2.8  | 3.3  | 4.4  | 5.4   | 6.1        |
| 2804              |                               | 2    | 1.2  | 2.1  | 2.6  | 3.4  | 4.2  | 4.7   | 5.7        |
| 2064              |                               | 1    | 1.5  | 2.5  | 3.4  | 4.0  | 5.0  | 5.9   | 7.0        |
| 2064              |                               | 2    | 1.5  | 2.2  | 2.8  | 3.5  | 4.6  | 5.1   | 6.0        |
| 1849              |                               | 1    | 2.0  | 3.1  | 3.8  | 4.4  | 5.5  | 6.1   | 7.1        |
| 1849              |                               | 2    | 1.8  | 2.5  | 3.3  | 4.2  | 5.1  | 6.1   | 7.0        |
Glass 3. Continued retardation.

Continued retardation is caused by wave-lengths: -
4960 A°, 3028 A°, 8804 A°, 2054 A° and 1849 A°. The growth
of the mycelium is stunted during the first few days after
irradiation, and the normal growth rate is never reached over
the period measured. It will be noted, however, that where
the initial stunting is not so intense the growth rate ap-
proximates more closely to normal (3804 A° and 1849 A°).

Class 4. No effect.

4359 A° does no affect the rate of growth to any
appreciable extent.

(See Plate 5.)

DISCUSSION OF RESULTS.

The effect of direct irradiation of growing cul-
tures and of suspension of spores in sterile water, on the
rate of growth of the organism can in both cases be seen to
be profoundly affected. It is apparent that the irradiation
of spores has a more marked effect than the irradiation of
the three day cultures, emphasizing the fact that the effect
must be purely protoplasmic, and not due to changes in the
culture medium caused by irradiation.

It will be seen from Plate 6 that when retard-
ation of growth extends over the ten day period, sporulation
The progressive effect of various lines of the mercury arc spectrum on the growth of Colletotrichum phomoides at time intervals of four and ten days.

Effect expressed as cm. increase or decrease over control culture growth.
The relations between the effect of monochromatic light on the growth and sporulation of Colletotrichum, the control represented as zero.
is hastened. It may be noted, however, that when the full mercury arc spectrum is used for irradiation, sporulation increased with exposures of from fifteen seconds to two minutes in duration, but decreased when exposures were for longer than two minutes. This seems to indicate that the time of sporulation is an inverse expression of growth rate only within certain limits and that there is an optimum amount of irradiation causing sporulation.

It will also be noted from Plate 6 that retardation and extreme stimulation of growth are accompanied by early sporulation, while irradiation causing intermediate stimulation of growth has no appreciable effect on the time of development of acervuli.

Stevens (34) working on various fungi found that irradiation "almost instantly initiates the development of reproductive structures in great numbers where they would not have occurred without irradiation". He suggests that these results may be due to a sudden inhibition of growth caused by the rays. On the other hand Ramsey and Bailey (30) irradiated Macrosporium and Fusarium and state that there is a definite stimulation of sporulation caused by ultra-violet light, which seems to be a direct result of stimulation rather than the indirect result of inhibition. Greatest sporulation was obtained at 2800 Å - 2535 Å. Stimulation of spore production occurred below 2535 Å but there was some lethal effect also and re-
tardation of mycelial development. These authors also found that long exposure to direct sunlight through filters trans­mitting no lower than 3132 A° induced abundant sporulation both in Macrosporium and Fusarium.

Fulton and Coblentz (8) irradiated the spores of many species of fungi with ultra violet light and found that the lethal effect resulted after comparatively short exposures, although the time required to kill varied with the type of fungus.

Dillon Weston (6) also reports that the primary reaction on Fusarium is that of retardation, subsequently, however, irradiated cultures sporulated very abundantly, whereas the non-irradiated cultures sporulated sparsely.

From these various experiments it will be seen that light has a profound effect on the production of spores, but whether this acceleration of spore production is the direct result of irradiation or the result of retardation of the mycelial growth causing a stimulation of spore production has not yet been determined.

Comparison of the effects of Monochromatic Light on animal protoplasm (Paramaecium) and on plant protoplasm (Colletotrichum)

In order to facilitate comparison we will deal with the results in classes, i.e., Class 1, those lines causing stimulation in Colletotrichum; Class 2, those lines
that give rise to retardation followed by stimulation in Colletotrichum and Class 3, lines that cause constant retardation in Colletotrichum.
(See Plate 7).

Class 1. 5819 A°, 3656 A°, 3342 A° and 4078 A° show continued stimulation in Colletotrichum. Of these 5819 A° and 3656 A° show similar stimulation in Paramoecium, 4078 A° giving stimulation followed by retardation.

Class 2. 6158 A°, 3132 A°, 3821 A° and 2967 A° give rise to retardation followed by stimulation in Colletotrichum. In Paramoecium the effects of the first three lines are not that of retardation but it will be noticed that after twenty-four hours the growth is more than that reported for the control, showing that the effect is one of less to more stimulation. 2967 A°, however, caused retardation in Paramoecium.

Class 3. 4960 A°, 3022 A°, 2804 A°, 2534 A° and 2054 A° are found to retard growth in both Paramoecium and Colletotrichum. Similar results were reported by Hutchinson and Newton (20) in their work on Yeast.

It can be seen from these results that there is a decided correlation between the two sets of results.
(Plate 7) In Class 3, where inhibition is reported in all cases it would seem that the far ultra violet has a profound effect on both animal and plant protoplasm and that certain regions in the visible spectrum, 4960 A°-4916 A°,
Diagram of the comparative effects of monochromatic light on Colletotrichum and Paramoecium. The control represented as zero.
<table>
<thead>
<tr>
<th>CLASS I</th>
<th>CLASS II</th>
<th>CLASS III</th>
</tr>
</thead>
<tbody>
<tr>
<td>5874 - 5769</td>
<td>3656 3342 4078</td>
<td>6152 3132 3821 2700</td>
</tr>
<tr>
<td>4960 - 4916</td>
<td>3022 2804 2535 2054 1849 A</td>
<td></td>
</tr>
</tbody>
</table>

**Legend**

- **Colletotrichum** (4 DA) □
- **10 DA** □
- **Paramaecium** (24 HRS) □
also exert an identical effect.

Where the effects are not so far-reaching, differences may be found between the two results, probably due to the varied susceptibilities of the organisms used and the difference in amount of light absorbed by the protoplasm.
SUMMARY OF RESULTS.

1. There are two regions in the Mercury Arc Spectrum that definitely inhibit the growth rate of Paramoecium caudatum: 4960 Å, 3132 Å and all shorter wave lengths used.

2. There are two regions of the Mercury Arc Spectrum that cause stimulation in Paramoecium caudatum growth rate: 5819 Å and 4359 Å.

3. Under the conditions of these experiments wave length and not intensity is the determining factor.

4. Slight transitory inhibition in growth rate is usually followed by a period of acceleration both in Paramoecium and Colletotrichum.

5. All lines of the Mercury Arc Spectrum used in these experiments caused an increase in the rate of plasmolysis of Paramoecium caudatum in Sodium Chloride solutions.

6. There is a definite correlation between division rate and plasmolytic rate in Paramoecium caudatum.

7. Irradiation of three day cultures of Colletotrichum phomoides caused retardation of growth and acceleration of sporulation when the full illumination from a Quartz Mercury Arc lamp was used.

8. Suspensions of spores of Colletotrichum phomoides irradiated with the Mercury Arc Spectrum had growth rate retarded and sporulation acceleration.
9. The effect of monochromatic light on spore suspensions is of three kinds, i.e., stimulation of growth, retardation followed by stimulation and retardation of growth.

10. Retardation or extreme stimulation of growth is accompanied by early sporulation, irradiation causing slight stimulation of growth having no apparent effect on development of acervuli.

11. A correlation can be seen between the effect of light on Paramoecium and on Colletotrichum, but it is impossible to say at this stage whether this holds for all animal protoplasm as compared with plant protoplasm.
PART B.

THE EFFECT OF RADIANT ENERGY ON ENZYME ACTION

INTRODUCTION

It would seem from results obtained in Part A of this work and in experiments undertaken by other authors, that light has a profound effect on both plant and animal protoplasm and that this effect must be based on one or more factors. What these factors are has yet to be determined. Several theories, however, have been propounded.

According to F. L. Gates (9) the effect of measured monochromatic ultra violet energy used to kill bacteria shows characteristic and similar curves at each wave length, but an appreciable amount of energy must be incident on the bacteria before any of them succumb, and widely different intensities of energy are required to produce these curves at different wave lengths. The reciprocals of these curves are similar to the absorption curves of certain derivatives of the nucleo-proteins which are related to cell growth and reproduction. This conclusion is supported by the known fact that the active agent of chicken tumours is associated with a nucleo-protein, which, in all cases of sarcomatous cells, gives a positive Feulgen reaction. It is therefore possible that the lethal effect and the inhibition of cell division caused by ultra
violet light may be related to its action on the nucleo-proteins of the nucleus.

F. I. Harris and H. S. Hoyte (13) prepared a pure culture of Paramecium and a second culture suspended in a solution of aromatic amino acids. The suspension was irradiated directly and the pure culture through a shield formed by a layer of the acid solution and in both cases the toxicity was decreased. This result suggested the possibility that the susceptibility of protoplasm to ultra-violet light is conditioned by the selective absorption of the toxic rays by the aromatic amino acid radicals of the proteins.

Hutchinson and Ashton (17) have suggested that resonance phenomena may be involved, and that certain particles in a colloidal or other state possess vibration periods such that their movements are affected by the specific frequencies of the radiant energy. On this basis subsequent effects may differ from the initial ones owing to associated changes in the colloidal condition.

As there is a certain dependance of enzyme activity on the colloidal state it was thought that the effect of radiant energy on enzyme action might throw some light on these basic factors causing lethal or stimulatory effects on protoplasm.
Light influences enzyme actions by destroying the enzyme itself or by modifying its activity. Lockman, Thies and Wiskern (22) found that the inhibitory effect of diffused light on peroxidase and blood catalase was in the following order, white > blue > red > dark. Reinle (31) found that the methylene blue formaldehyde reductase reaction of cow's milk was reduced by the action of ultra violet light. Getchell and Walter (10) stated that ultra violet light was more potent in the destruction of peroxidase activity than either X-rays, radium or visible light.

Pincussen (87) found that the action of light on diastase and urease was dependent on the dilution of the enzyme, the various impurities present and upon the reaction. Radium radiations were also found to be injurious to rennet but to have a stimulative effect on diastase. X-rays also have varied effects on enzymes, the diastatic activity of urine, serum and various fluids being unchanged while that of pepsin and amylase was considerably altered.

H. J. Fuller (7) experimented with plant tissue injured by ultra violet radiation in respect to catalase and diastase activity and found that the activity of these enzymes actually increased to a considerable extent in tissues exposed to destructive wave lengths and that there was no evidence whatsoever of a diminution of enzyme
activity, a condition contrary to the results of previous investigators. This author believes that the injurious effects of ultra violet light must be traceable to some physiological derangement other than the inactivation of enzymes.

It would seem that various forms of radiant energy have varying effects on enzymes from different sources and the work reported here is an attempt to find some correlation between the effect of light on plant enzymes and on animal enzymes in the hope that some definite classification might be found and that some connection might be established between these results and the results previously reported on the effect on growth and reproduction.

EXPERIMENTAL WORK.

PART I. Effect of Radiant Energy on Diastase.

PART II. Effect of Radiant Energy on Oxidase Activity of Potato extract.

PART I.

The most widely distributed enzymes that control the breaking down of the higher carbohydrates are the Diastases (Amylases). These enzymes break down carbohydrates to an end product of maltose with the intermediate production of erythro and achroo-dextrins. In these experiments the
animal amylase used was Ptyalin, the diastatic enzyme present in human saliva. The plant enzyme used was malt Diastase, from germinated barley.

The divergence in results obtained during the breaking down of starch by diastase of various origins has led to the belief in the presence of two enzymes, a saccharogenic enzyme and a starch liquifying enzyme. Sherman and Schlesinger (33) and Kendal and Sherman (21) have demonstrated that the liquifying action is predominant in pancreatic amylase and the saccharifying in malt diastase, and have also shown that the liquifying enzyme is predominant in salivary diastase. These workers found that the proportion of the two actions was usually constant for each particular enzyme although great differences in proportion were noticeable in enzymes of varied sources, this difference being ascribed "to the presence of complements or activators which are required for splitting up of certain definite atomic groups".

Now it was thought that light, having a definite effect on enzyme action, might have a varying effect on the two enzymes present in Diastase, acting as an inhibitor or activator of the different groups. The experiments here described were therefore carried out on the amylolytic action of diastase as distinct from the saccharogenic action, but it is hoped in the near future to ascertain the effect on the sugar forming enzyme and to find whether the differences
are limited to either plant or animal amylase or possessed by both.

MATERIALS

1. Malt Diastase. .75 grms. of Malt Diastase were shaken for 15 minutes in 250 c.c. distilled water and then filtered. This solution plus two drops of toluene as a preservative was kept in the dark in a stoppered bottle.

2. Salivary diastase. Samples of saliva were obtained thus:— the mouth was washed out with distilled water at 40°F. 20 c.c. of water was then held in the mouth for one minute and collected. This was repeated twice. The 40 c.c were then shaken, filtered and the resulting solution kept in the dark. It was found that the activity of the solution became less on the third day so that a fresh solution was made up every three days and was found to be remarkably constant in its activity. The achromic point of the saliva was ascertained and if it varied greatly from four minutes adjustment was made of the concentration of the solution to obtain an achromic point of constant value.

3. Substrate used. C. P. soluble starch was used throughout all the experiments.

Solutions of starch were made up of the following concentrations:— 0.1%, 0.2%, 0.3%, 0.5%, 0.75%, 1.0%,
In the case of Salivary Diastase 25 c.c. of a phosphate buffer of 6.7 pH were used to maintain the pH maximum at 6.7, which is the optimum pH for ptyalin activity. 25 c.c. of 1% NaCl were also added as the action of animal amylase has been proved to be conditioned by the presence of neutral salts in quantities varying from .02% to 2.0%.

The 0.1% starch solution would therefore be made up as follows:

- 4 c.c. 5% starch solution.
- 25 c.c. Buffer
- 25 c.c. 1% NaCl
- 146 c.c. Distilled water.

The other solutions being made up with the required proportions of starch and distilled water.

In the case of the Malt Diastase the same concentrations of starch solution were made up, but the pH was kept between 4.8 and 5.2 by means of a phthalate buffer. The 0.1% starch solution being made up as follows:

- 4 c.c. 5% starch solution
- 25 c.c. Buffer
- 171 c.c. distilled water.

**PROCEDURE**

3 c.c. of enzyme solution were placed in each of two quartz tubes. One tube was fitted into the slit of the monochromatic illuminator or irradiated directly in front of the quartz mercury lamp and the other was
kept in the dark as a control.

In the case of salivary diastase 1 c.c. of the irradiated solution was diluted to 100 c.c. with distilled water and 1 c.c. of this solution placed in each of the series of test tubes containing 5 c.c. of each of the starch solutions. Thus 0.01 c.c. of enzyme was present in each test tube plus 5 c.c. starch solution. The same process was repeated for the control. One drop of toluene was added to each test tube, which was then shaken, and placed in an oven at 38°-40°F.

3 drops of the irradiated malt diastase were added by means of a dropping pipett to the starch series with 2 drops of toluene per test tube, shaken, and placed in the oven at 38°-40°F. Three drops of the non-irradiated control also being added to another series and that in turn placed in the oven.

At stated intervals of time one drop was taken from each of the series of test tubes, irradiated and control, placed on a glass plate, using a dropping pipette, and one drop of M/10 Iodine added. The first appearance of red colouration, denoting conversion of starch to dextrin, was noted and the number of the test tube recorded.
RESULTS.

Salivary Diastase.

1. The effect of full illumination from a quartz mercury lamp on the amylolytic action of saliva.

a). Three c.c. of enzyme were irradiated at various distances from a quartz mercury lamp for a period of one hour and the achromatic point of the solution ascertained. (Table 6.) These results show that the activity of the enzyme is greatly reduced by the action of light in direct proportion to the distance from the source.

b). Three c.c. samples of salivary diastase were irradiated for one hour at distances of 10, 7, 5 and 3 cms. from the source of light. The effect of the various irradiated enzyme solutions on the series of starch tubes as previously described was ascertained at varying periods of time, and a comparison made with the control. (Table 7, Plate 8).

It will be seen that the full light from a mercury arc lamp has a decided inhibitory action on the activity of salivary diastase, evidently in proportion to the intensity of the light. The rise in temperature of the extracts so irradiated was negligible as compared with the control except in the case of irradiation at a distance of 3 cms. from the source of light when the temperature was raised about 3°C above room temperature. The almost total
<table>
<thead>
<tr>
<th>Distance from Light Source</th>
<th>Achromic Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 cms.</td>
<td>4 mins.</td>
</tr>
<tr>
<td>20 cms.</td>
<td>6 mins.</td>
</tr>
<tr>
<td>10 cms.</td>
<td>12 mins.</td>
</tr>
<tr>
<td>7 cms.</td>
<td>36 mins.</td>
</tr>
<tr>
<td>5 cms.</td>
<td>64 mins.</td>
</tr>
<tr>
<td>3 cms.</td>
<td>No end point.</td>
</tr>
</tbody>
</table>

**TABLE 7.**

The effect of full illumination from a quartz mercury lamp on the activity of Salivary Diastase.

Activity of enzyme expressed as amount of starch reduced. (Percentage concentration of solution).

<table>
<thead>
<tr>
<th>Hours</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>24</th>
<th>48</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>.5</td>
<td>1.0</td>
<td>1.5</td>
<td>-</td>
<td>-</td>
<td>2.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.5</td>
<td>3.0</td>
<td>4.0</td>
<td>5.0</td>
</tr>
<tr>
<td>10 cms.</td>
<td>.5</td>
<td>.75</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
<td>1.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.0</td>
<td>2.5</td>
<td>3.0</td>
<td>4.0</td>
</tr>
<tr>
<td>7 cms.</td>
<td>.2</td>
<td>-</td>
<td>.5</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.5</td>
<td>2.0</td>
<td>2.5</td>
<td>3.0</td>
</tr>
<tr>
<td>5 cms.</td>
<td>.2</td>
<td>-</td>
<td>-</td>
<td>.3</td>
<td>-</td>
<td>-</td>
<td>.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>.75</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>3 cms.</td>
<td>.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

.................
Effect of full illumination from a quartz mercury lamp on the amylolytic action of saliva and of malt diastase.

Ordinates. Enzyme activity expressed as percentage concentration of starch solution reduced.

Abscissae. Time after cessation of irradiation.

A. Effect on Salivary Diastase.

B. Effect on Malt Diastase.
inactivation of the enzyme so irradiated may be therefore partly due to the destructive action of the wave lengths of the longer part of the spectrum.

It is of interest to note that an experiment was carried out to ascertain the approximate effect of full illumination on the amount of reducing sugar formed and the effect was proved to be decidedly inhibitory in nature.

2. The specific effects of Monochromatic Light on Salivary diastase.

Taking these results in classes we have:

Class 1. Wave lengths having no effect.

These include 6152 A°, 5619 A° and 3821 A°, which exert no measurable effect on the activity of the enzyme.

Class 2. Wave lengths having a stimulating effect.

4359 A° and 4078 A° appear to have a slight stimulatory effect, which is lost, however, after some hours.

3663 A° on the other hand exerts a slight inhibitory effect at first, followed by a stimulation which is maintained throughout the experiment.

Class 3. Wave lengths causing a retardation in action.

5461 A°, 4916 A°, 3132A° with all lines of shorter wave length possess a decided inhibitory effect, which is maintained throughout the period. In the case of 3022 A° the inhibition is only initial and full recovery takes place by the completion of the experiment.
Table 8.

The specific effects of Monochromatic Light on Salivary Diastase.

Activity of the enzyme expressed as amount of starch reduced, percentage concentration of solution.

Readings after twenty-four hours irradiation.

<table>
<thead>
<tr>
<th>Hours</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>24</th>
<th>48</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.5</td>
<td>1.0</td>
<td>1.5</td>
<td>-</td>
<td>-</td>
<td>2.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.5</td>
<td>3.0</td>
<td>4.0</td>
<td>5.0</td>
</tr>
<tr>
<td>6152A°</td>
<td>No effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5819A°</td>
<td>No effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5461A°</td>
<td>0.3</td>
<td>0.5</td>
<td>0.75</td>
<td>1.0</td>
<td>-</td>
<td>1.5</td>
<td>-</td>
<td>2.5</td>
<td>3.0</td>
<td>4.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4916A°</td>
<td>0.3</td>
<td>0.5</td>
<td>0.75</td>
<td>1.0</td>
<td>1.5</td>
<td>-</td>
<td>-</td>
<td>2.0</td>
<td>2.5</td>
<td>3.0</td>
<td>4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4359A°</td>
<td>0.75</td>
<td>1.0</td>
<td>1.5</td>
<td>-</td>
<td>2.0</td>
<td>-</td>
<td>-</td>
<td>2.0</td>
<td>3.0</td>
<td>4.0</td>
<td>5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4078A°</td>
<td>0.75</td>
<td>1.0</td>
<td>1.5</td>
<td>-</td>
<td>2.0</td>
<td>-</td>
<td>-</td>
<td>2.0</td>
<td>3.0</td>
<td>4.0</td>
<td>5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3821A°</td>
<td>No effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3663A°</td>
<td>0.5</td>
<td>1.0</td>
<td>1.5</td>
<td>-</td>
<td>2.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.0</td>
<td>4.0</td>
<td>5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3132A°</td>
<td>0.3</td>
<td>0.3</td>
<td>0.5</td>
<td>0.75</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
<td>2.0</td>
<td>3.0</td>
<td>4.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3022A°</td>
<td>0.3</td>
<td>0.5</td>
<td>0.75</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.0</td>
<td>4.0</td>
<td>4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2967A°</td>
<td>0.3</td>
<td>0.5</td>
<td>0.75</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.5</td>
<td>3.0</td>
<td>4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2804A°</td>
<td>0.3</td>
<td>0.5</td>
<td>0.75</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
<td>2.5</td>
<td>3.0</td>
<td>4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2700A°</td>
<td>0.5</td>
<td>0.5</td>
<td>0.75</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.0</td>
<td>3.0</td>
<td>4.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2535A°</td>
<td>0.3</td>
<td>0.5</td>
<td>0.75</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td>1.5</td>
<td>2.0</td>
<td>2.5</td>
<td>3.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Results  Malt Diastase.

1. The effect of full illumination from a quartz mercury lamp on the activity of malt diastase.

A decided inhibitory effect is recorded, increasing with the intensity of the light, the greatest inhibition being found when the solutions were irradiated at 3 cms. distant from the source of light. (Plate 8)

2. The specific effects of monochromatic light on the activity of malt diastase.

It was found that only four wave lengths, i.e., 5461 Å, 4916 Å, 3132 Å and 3022 Å had any effect on the diastatic action of the malt solution. The lack of effect in the shorter wave lengths may be due largely to the fact that the intensity of the incident light was not sufficient to effect any change in enzyme action, although it must be noted that 3132 Å, 3022 Å and 4916 Å are all lines of very low intensity, especially as compared to 5461 Å, and all exert a decidedly inhibitory action, pointing to the fact that intensity is not the controlling factor in this case.

COMPARISON OF RESULTS.

1. In both cases, that of salivary and malt diastase full illumination from a quartz mercury lamp has a decided inhibitory effect on the activity of these enzymes. In the case of saliva the most intense illumination used
### TABLE 9.

**Effect of full illumination from a quartz mercury lamp on the activity of Malt Diastase.**

Enzyme activity expressed as amount of starch reduced.

**Readings after twenty-four hours irradiation.**

<table>
<thead>
<tr>
<th>Days</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>1.5</td>
<td>2.0</td>
<td>-</td>
<td>2.5</td>
<td>-</td>
<td>3.0</td>
<td>-</td>
<td>4.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10 cm</td>
<td>1.0</td>
<td>1.5</td>
<td>-</td>
<td>-</td>
<td>2.0</td>
<td>-</td>
<td>-</td>
<td>3.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7 cm</td>
<td>1.0</td>
<td>1.5</td>
<td>-</td>
<td>-</td>
<td>2.0</td>
<td>-</td>
<td>-</td>
<td>2.5</td>
<td>3.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5 cm</td>
<td>.75</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
<td>1.5</td>
<td>-</td>
<td>-</td>
<td>2.5</td>
<td>3.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5 cm</td>
<td>.3</td>
<td>.5</td>
<td>-</td>
<td>.75</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
<td>1.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

### TABLE 10.

**Specific effects of monochromatic light on the activity of Malt Diastase.**

<table>
<thead>
<tr>
<th>Control</th>
<th>1.5</th>
<th>2.0</th>
<th>-</th>
<th>2.5</th>
<th>-</th>
<th>3.0</th>
<th>-</th>
<th>4.0</th>
<th>-</th>
<th>-</th>
<th>-</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>5461A°</td>
<td>1.5</td>
<td>-</td>
<td>-</td>
<td>2.0</td>
<td>-</td>
<td>-</td>
<td>2.5</td>
<td>-</td>
<td>3.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4916A°</td>
<td>1.5</td>
<td>-</td>
<td>-</td>
<td>2.0</td>
<td>-</td>
<td>2.5</td>
<td>-</td>
<td>3.0</td>
<td>-</td>
<td>4.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5132A°</td>
<td>1.5</td>
<td>-</td>
<td>-</td>
<td>2.0</td>
<td>-</td>
<td>-</td>
<td>2.5</td>
<td>-</td>
<td>3.0</td>
<td>-</td>
<td>4.0</td>
<td>-</td>
</tr>
<tr>
<td>3022A°</td>
<td>1.5</td>
<td>-</td>
<td>-</td>
<td>2.0</td>
<td>-</td>
<td>2.5</td>
<td>3.0</td>
<td>-</td>
<td>4.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
40.

(30 cm. distant) has an almost completely paralysing effect in contrast to malt diastase, which shows gradual recovery from the effects of irradiation at that distance (Plate 8).

It must be remembered, however, that the normal activity of the two amylases is very different, the starch liquifying enzyme in saliva being many times more rapid in its action than the liquifying portion in malt diastase. These results may be in part attributable to this difference in activity, the least active enzyme being the least affected.

2. The results from illumination with monochromatic light are interesting in that four lines, 5461 Å, 4916 Å, 3132 Å and 3022 Å in all cases exert a decidedly inhibitory action on the activity of the enzymes. That this is due solely to wave length and not to intensity can be clearly seen by comparing the relative intensities of 5461 Å and 4916 Å, lines which exert, especially in the case of Malt diastase, practically the same amount of inhibition with very unequal intensities.

The wave lengths that cause stimulation in salivary diastase have no effect on Malt diastase, i.e., 4359 Å, 4078 Å and 3663 Å.

In salivary diastase all wave lengths shorter than 3022 Å exert a decidedly inhibitory effect on the enzyme activity but have no effect on that of malt diastase.
Comparison of the effect of monochromatic light on the growth of *Paramoecium caudatum* and on the activity of animal diastase.

*Paramoecium* - increase or decrease in number of cells after twenty-four hours irradiation over the control count.

Enzyme activity expressed as percentage concentration of starch solution reduced at periods of three and twenty-four hours after irradiation.
Comparison of the effect of ultra violet light on growth with the effect on enzyme action.

1. Comparison of the effect of Monochromatic light on growth of animal protoplasm and on the activity of animal diastase. (Plate 9.)

There appears to be some correlation between the results of these experiments. The line causing the greatest stimulation of growth in Paramocelium, i.e., 3655 Å, shows the maximum stimulatory effect on the activity of salivary diastase. Those lines that only slightly stimulate growth appear to have no effect on the activity of the enzyme. The shorter wave lengths, causing an inhibition of growth in Paramocelium, exert a decided inactivating effect on the enzyme. 4078 Å, causing inhibition in growth does not effect the enzyme activity and 3122 Å, having no effect on growth, has an inactivating effect on enzyme action, but apart from these two lines giving divergent results there appears to be a correlation between growth rate and diastatic activity under the conditions of these experiments. (Plate 9.)

2. Comparison of the effect of monochromatic light on the growth of plant protoplasm and on the activity of plant diastase.

There seems to be no evident correlation between the results obtained for malt diastase and those on the
growth and sporulation of Colletotrichum, although 4916 Å and 3022 Å cause inhibition in all cases. This lack of correlation is perhaps due to the fact that malt diastase is an enzyme from a plant containing chlorophyll and Colletotrichum is a fungus. There may be a difference between the sensitivity of enzymes in photosynthetic plants and in those lacking chlorophyll. Experiments are to be undertaken on enzymes obtained from fungi in order to clarify this point.

Sufficient data has not yet been obtained to enable any definite statement to be made, but the results so far point to the fact that plant diastase is less sensitive to light of various wave lengths than animal diastase.

Potato extract was used in the following experiments to determine the effect of ultra violet light and monochromatic light on oxidase activity.

The method of measuring the oxidase activity was based on that outlined by Guthrie (12.) He noted that potato juice contained a substance that might be titrated with iodine in acid solution (trichloracetic acid) and that this titration value decreased on exposure to air. However, in some cases the titration was found to be very much increased and it was believed to be due to a low content of oxidase, the "substance responsible for the iodine reaction not being oxidised in the process of extraction for this reason". Guthrie found that glucose warmed with dilute NaOH contained a carbohydrate derivative capable of reducing iodine in acid solution. This titration decreased on exposure to air and the oxidation was catalysed by potato juice. It would therefore be possible to measure the activity of the oxidase in the potato juice by the amount of iodine reduced by juice and substrate.

METHODS.

1. Preparation of substrate.

40 grms. of glucose were dissolved in 400 c.c. of Normal sodium hydroxide, placed in a 500 c.c. flask and immersed
in a water-bath at 80°C for 15 minutes. The solution was removed and neutralised at once by adding 10 c.c. of 85% phosphoric acid. 25 grms. of decolourizing charcoal was added and the mixture allowed to stand overnight. The mixture was then filtered and another 25 grms. of charcoal added and allowed to stand for 15 minutes and again filtered. The pH was then adjusted to 6.5 by means of N/10 sodium hydroxide or hydrochloric acid.

2. Preparation and irradiation of enzyme extract.

An extract of potato juice containing the enzyme was obtained by grinding up a measured amount of potato and squeezing the ground material through cheese cloth with a potato masher. 3 c.c. of the extract so obtained was placed in a quartz container, stoppered with cotton wool, and either irradiated by the full illumination from a quartz mercury lamp or placed in the slot of the monochromatic illuminator, for varying periods of time.


20 c.c. of the substrate diluted 1:1 with distilled water was placed in each of two aeration tubes, A and B. To A was added 3 c.c. of potato extract which had been kept in the dark during the irradiation period to act as a control. To B was added 3 c.c. of irradiated extract. 5 drops of paraffin oil were added to each to act as a foam breaker, and both A and B were aerated for one hour with equal currents of air. At the end
of one hour 5 c.c. from each A and B were washed into each of four flasks containing 5 c.c. of 10% trichloracetic acid and 10 c.c. of Iodine. (N/50 I in N/10 KI). After five and ten minutes, two flasks, control and irradiated respectively were titrated with N/100 Sodium thiosulphate using 1 c.c. of 1% starch paste as an indicator. The difference between the titrations of A and B was used as a measure of the difference between the oxidase activity of the control and the irradiated sample, a larger titration in B (irradiated) denoting a greater oxidase activity as compared to the control (A), and vice versa.

EXPERIMENT I

Irradiation with the full illumination from a quartz mercury lamp.

3 c.c. portions of potato extract were irradiated:

1. in glass containers.
2. in quartz containers.

at a distance of 10 cms. from the source of light, for varying periods of time, the temperature being 22°C, room temperature.

Results.

1. Irradiation in glass containers.

(Table 11.)

It is probable that the glass of these containers absorbs all wave lengths less than about 3300 Å, so that any effect on the oxidase activity would be caused by the
**TABLE II**

**EFFECT of illumination from the rays of a quartz mercury lamp on oxidase activity of potato juice.**

Distance from source 10 cms.

Oxidase activity expressed as % increase or decrease over control titrations.

A. **Irradiated in glass containers.**

<table>
<thead>
<tr>
<th>Time of Irradiation</th>
<th>Readings after 5 minutes</th>
<th>Readings after 10 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hour</td>
<td>-5.7</td>
<td>-4.6</td>
</tr>
<tr>
<td>2 hours</td>
<td>1.5</td>
<td>5.7</td>
</tr>
<tr>
<td>3 hours</td>
<td>10.3</td>
<td>10.6</td>
</tr>
<tr>
<td>4 hours</td>
<td>13.6</td>
<td>13.1</td>
</tr>
<tr>
<td>5 hours</td>
<td>5.4</td>
<td>4.3</td>
</tr>
<tr>
<td>6 hours</td>
<td>4.6</td>
<td>4.0</td>
</tr>
<tr>
<td>7 hours</td>
<td>3.6</td>
<td>3.5</td>
</tr>
</tbody>
</table>

B. **Irradiated in Quartz containers.**

<table>
<thead>
<tr>
<th>Time of Irradiation</th>
<th>Readings after 5 minutes</th>
<th>Readings after 10 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hour</td>
<td>-4.2</td>
<td>- 2.1</td>
</tr>
<tr>
<td>2 hours</td>
<td>-13.8</td>
<td>-10.8</td>
</tr>
<tr>
<td>3 hours</td>
<td>- 6.2</td>
<td>- 3.9</td>
</tr>
<tr>
<td>4 hours</td>
<td>- 4.8</td>
<td>- 3.7</td>
</tr>
<tr>
<td>5 hours</td>
<td>- 3.5</td>
<td>- 3.8</td>
</tr>
<tr>
<td>6 hours</td>
<td>- 3.4</td>
<td>- 3.8</td>
</tr>
<tr>
<td>7 hours</td>
<td>- 3.2</td>
<td>-3.6</td>
</tr>
</tbody>
</table>
Effect of illumination from a mercury arc lamp on the oxidase activity of potato extract.

A. Illumination through a glass container.

B. Illumination through a quartz container.

Oxidase activity expressed as percentage increase or decrease over the control titrations.
visible and some of the near ultra violet wave lengths. It would seem from Plate 10 that the enzyme activity was decreased at first, but after one hour's irradiation it was distinctly increased as compared to the control at that time, and that after seven hours irradiation was still more active than the control extract. The maximum activity being shown after approximately three hours irradiation.

2. Irradiation in quartz containers.

These quartz vessels do not absorb any of the far ultra violet lines until around 2500 Å. It will be seen that at all periods of irradiation the oxidase activity was decreased, reaching a minimum at around the two hours and then gradually returning nearer to the control. But after a period of eighteen hours exposure the decrease was very marked. It would seem from the above results that the far ultra violet lines have an inhibitory effect on oxidase activity sufficient to counteract any stimulation caused by the wave lengths of the visible and near ultra violet. The point of maximum effect seemed to be around 3-4 hours with the glass and two to three hours with the quartz containers.

EXPERIMENT 2. Effect of monochromatic light on oxidase activity of potato extract.

3 c.c. of potato extract were placed in a quartz container in the slot of a monochromatic illuminator for
periods of three and in some cases eighteen hours and subjected to the various monochromatic lines of the mercury arc spectrum. 3 c.c. of the same extract were placed in the dark, at the same temperature, to act as a control.

RESULTS.

Class I. Stimulation

6152 A° causes a considerable increase in activity which, is however, decreased after 18 hours irradiation due probably to the equilibrium being reached more quickly than in the control. 5819 A° causes an increase in activity maintained throughout the eighteen hours, and 5461 A° and 4960 A° result in the same increase in activity. 4359 A° and 3821 A° cause only a slight increase in activity maintained throughout the whole period.

Class II.

4359 A°, 4078 A° seem to have but slight effect, which may be covered by experimental error, but in no case are the effects entirely negative.

Class III.

3132 A°, 3022 A°, 2804 A° and 2700 A° cause decided and continued decrease in activity. 2535 A°, 2064 A°, both approaching the limits of transition of quartz glass, cause slight but continued decrease in activity.
TABLE 12.

Specific effects of monochromatic light.

Oxidase activity expressed as % increase or decrease over control titrations.

<table>
<thead>
<tr>
<th>Wave length in Å</th>
<th>Time Irrad.</th>
<th>Readings after 5 minutes</th>
<th>Readings after 10 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>6152</td>
<td>3 hrs.</td>
<td>3.3</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>18 hours</td>
<td>-2.2</td>
<td>-1.4</td>
</tr>
<tr>
<td>5619</td>
<td>3 hours</td>
<td>1.4</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>18 hours</td>
<td>1.6</td>
<td>1.4</td>
</tr>
<tr>
<td>5461</td>
<td>3 hours</td>
<td>1.3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>18 hours</td>
<td>2.9</td>
<td>5.2</td>
</tr>
<tr>
<td>4960</td>
<td>3 hours</td>
<td>1.3</td>
<td>*6</td>
</tr>
<tr>
<td></td>
<td>18 hours</td>
<td>2.6</td>
<td>2.0</td>
</tr>
<tr>
<td>4359</td>
<td>3 hours</td>
<td>*6</td>
<td>0</td>
</tr>
<tr>
<td>4078</td>
<td>3 hours</td>
<td>*6</td>
<td>*6</td>
</tr>
<tr>
<td>3821</td>
<td>3 hours</td>
<td>1.2</td>
<td>*6</td>
</tr>
<tr>
<td>3132</td>
<td>3 hours</td>
<td>-1.3</td>
<td>-1.3</td>
</tr>
<tr>
<td></td>
<td>18 hours</td>
<td>-2.4</td>
<td>-2.4</td>
</tr>
<tr>
<td>3022</td>
<td>3 hours</td>
<td>-1.9</td>
<td>-11.3</td>
</tr>
<tr>
<td></td>
<td>18 hours</td>
<td>-3.7</td>
<td>-11.3</td>
</tr>
<tr>
<td>2804</td>
<td>3 hours</td>
<td>-2.4</td>
<td>-2.5</td>
</tr>
<tr>
<td>2700</td>
<td>3 hours</td>
<td>-3.0</td>
<td>-3.0</td>
</tr>
<tr>
<td>2536</td>
<td>3 hours</td>
<td>-1.8</td>
<td>*6</td>
</tr>
<tr>
<td>2054</td>
<td>3 hours</td>
<td>-1.6</td>
<td>*5</td>
</tr>
</tbody>
</table>

..........................
DISCUSSION OF RESULTS.

From the results just recorded it would seem that visible and ultra violet light have an appreciable effect on the oxidase activity of potato extract under the conditions of these experiments.

Visible light, especially the yellow portion of the spectrum, causes a stimulation, although the transition lines around 4078 Å show a slight decrease in activity. The near ultra violet lines cause a decrease in activity which becomes greater as the far ultra violet is approached.

The total effect of the lines of the Mercury Arc Spectrum causing decrease in activity seems to be greater than that caused by the stimulating lines, as is shown when irradiation is carried out in glass as compared to quartz containers. In the case of the quartz containers, which do not absorb light longer than about 2500 Å, continuous inhibition is reported, whereas when glass containers, cutting out some of the near and all of the far ultra violet, are used, stimulation is found to occur in practically all cases.
SUMMARY OF RESULTS.

1. The activity of salivary and malt diastase is reduced by the action of light from a mercury arc lamp in proportion to the intensity.

2. The effects of monochromatic light on the activity of salivary diastase are of two kinds, stimulatory and inhibitory. 4359 Å, 4078 Å, and 3663 Å exerting stimulation and 5461 Å, 4916 Å, 3132 Å, and 3022 Å exerting an inhibitory effect.

3. Four lines only of the mercury arc spectrum exert any effect on the activity of malt diastase, i.e., 5461 Å, 4916 Å, 3132 Å, and 3022 Å. This effect is one of inhibition.

4. Under the conditions of these experiments there appears to be some correlation between the effect of the monochromatic lines of the mercury arc spectrum on the growth of Paramecium and on the activity of salivary diastase; those lines causing stimulation in growth in most cases causing increase in enzyme action and those lines causing inhibition of growth decreasing the activity of the enzyme.

5. There appears to be no correlation between the results obtained for malt diastase and for growth of Colletotrichum.

6. There are indications that under the conditions of these experiments plant diastase is less sensitive to light from the mercury arc spectrum than is animal diastase.
7. The visible and near ultra violet lines of the mercury arc spectrum cause an increase in the oxidase activity of potato extract.

8. The far ultra violet cause a decrease in oxidase activity of potato extract.

9. The total effect of the lines of the mercury arc spectrum causing inhibition of oxidase activity seems to be greater than that of the lines causing increase in activity.


33. Sherman, H. C. and Schlesinger, M. D. Comparison of amyelolytic and saccharogenic powers of various diastases.

