CHAPTER III

EFFECT OF ULTRAVIOLET RADIATIONS UPON CELLS

A. EFFECT OF RADIATIONS UPON CHEMICAL REACTIONS

It has long been known that energy in the form of visible light, or near this range, will produce chemical changes, which we term photochemical reactions. Just as a chemical synthesis may be brought about by heat, so may it be induced by adding radiant energy in the form of light. All organic matter is thus produced from CO_2 , H_2O and nitrates, by means of radiant energy from the sun, with chlorophyl as the necessary "transformer" of the energy.

It is not necessary, however, that radiant energy be present to increase the energy level of each reacting molecule. A number of chemical reactions are known which require some radiant energy to be initiated; however, several hundred, or even several million molecules are changed for each energy quantum which is absorbed. These reactions are exothermic. If only one molecule becomes activated by an energy quantum of the right size, the energy liberated by its reaction activates another molecule. Thus, many molecules may be changed though it is quite impossible that one quantum can be absorbed by more than one molecule. This type of reaction, which is called a *chain reaction*, is usually explained in the following way (H. S. TAYLOR, 1931, p. 1009—1018):

The quantum, in being absorbed by a molecule, ionizes it. The ion pair thus produced initiate two independent reactions which again produce ions. The classical example by BODENSTEIN and NERNST is the photochemical reaction between the gases H_2 and Cl_2 . The chain reaction can be written

| $\mathrm{Cl}_2 + \mathrm{h}\nu = \mathrm{Cl} + \mathrm{Cl}$ | | | | | | | | |
|-------------------------------------------------------------|--------------------------------------------------------------------|--|--|--|--|--|--|--|
| $Cl + H_2 = HCl + H$ | $Cl + H_2 = HCl + H$ | | | | | | | |
| $H + Cl_2 = HCl + Cl_2$ | $\mathbf{H} + \mathbf{Cl}_2 = \mathbf{H}\mathbf{Cl} + \mathbf{Cl}$ | | | | | | | |
| $Cl + H_2 = HCl + H$ | $Cl + H_2 = HCl + H$ | | | | | | | |
| $H + Cl_2 = HCl + Cl_2$ | $\mathbf{H} + \mathbf{Cl}_2 = \mathbf{H}\mathbf{Cl} + \mathbf{Cl}$ | | | | | | | |
| $Cl + H_2 = HCl + H_2$ | | | | | | | | |
| $\dot{\mathbf{H}} + \dot{\mathbf{Cl}} = \mathbf{HCl}.$ | | | | | | | | |

Under "chain" is understood the number of consecutive molecules entering into reaction. The chain may be as "long" as one million molecules. The "length" can be ascertained by determining the number of molecules changed for each quantum of light entering the system. The chain is terminated by the reactive molecules or atoms combining with each other, as indicated in the above model, or by reacting with other molecules to form stable compounds.

If it were not for these terminations, one quantum would be sufficient to cause all molecules to react with one another. In fact, in the case of explosions, where the reaction liberates a large amount of energy, this is practically the result.

The presence of foreign substances reacting with the components of the system is a common cause of cessation. If there were only one such molecule present for every million molecules of hydrogen and chlorine, that would account for an average chain length of one million molecules. If there were 100 times as much of the foreign substance, the chain length would be reduced to ten thousand molecules.

The same reasoning holds true for chain reactions in solutions. In the photochemical oxidation of Na_2SO_3 to Na_2SO_4 , the quantum yield was about 100000. This reaction was inhibited by primary and secondary, but not by tertiary alcohols. Whenever a chain was terminated, two molecules of the alcohols were oxidized to the corresponding aldehydes and ketones.

The known chain reactions are exothermic. It does not seem imperative, however, that they be so if another source of energy is available. This is the case in normally nourished cells of animals, fungi, bacteria, etc., which liberate energy constantly by metabolizing carbohydrates, fats, proteins or other organic compounds. By means of this energy, they grow, i. e. they synthetize new body substance endothermically.

It is not impossible, that very small amounts of energy, even a single quantum, might produce a very noticeable effect in a living cell. This could be accomplished by releasing a complex mechanism which needs only one or several quanta of a given size to be initiated, just as the Cl_2 -molecule needed only one quantum of the right size to start the reaction with hydrogen. The most common, and perhaps the only reaction in the cell which can be thus released is cell division; this results in a more rapid multiplication, or an increased growth rate.

B. EFFECT OF MONOCHROMATIC ULTRAVIOLET UPON LIVING CELLS

Ultraviolet light of any of the different physical sources mentioned in the previous chapters may have a very distinct effect upon living cells. The best-known is the reddening of the skin by ultraviolet light. A quantitative study of the relation between the intensity of the effect and the wave length has revealed that aside from the very marked erythema produced by wavelengths around 3000 Å, the cells of the skin will also react upon those below 2600 Å which are not found in sunlight. Between these two maxima is a zone of very weak effects. This fact is significant because the major part of this book is concerned with mitogenetic rays which are shorter than 2600 Å.

Ultraviolet light also kills bacteria and other microorganisms. Strangely, however, the intensity curve for the different wavelengths is quite different from that of the erythema effect, it looks almost like the reverse. Figure 27 shows the results by COBLENTZ, STAIR and HOGUE (1932) on erythema, by RIVERS and GATES (1928) on vaccine virus and Staphylococcus aureus and by DUGGAR and HOLLAENDER (1934) on Bact. prodigiosum and the mosaic virus of tobacco. Most of these investigations have been carried out no further than to a wave length of about 2500 Å.

If shorter wave lengths are taken into consideration, and especially if the intensity is very greatly decreased, it is possible



Figure 27. Comparative intensities of the killing effect of different wave lengths. The intensities are uniform for each individual organism, but vary greatly for the different curves.

to obtain growth stimulation under certain conditions which will be specified in Chapter IV. The result of the most extensive of the many experiments of this nature is shown graphically in fig. 28.



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CHARITON, FRANK and KANNEGIESSER (1930) used individual spectral lines, employing a monochromator, from the sparks of aluminum, zine or cadmium, to irradiate yeast cultures. Each culture thus obtained light of one definite wave length. By varying the intensity as well as the wave length, it could be shown that only radiation of less than 2700 Å produced positive effects, i. e. increased the growth rate of yeast.

A large number of similar experiments have been carried out by GURWITSCH and his associates, usually as controls for organic radiations. They will be mentioned in the succeeding chapters.

There seems to be very little difference in the limiting intensities of different wave lengths. Other experiments have shown that even at 1900 Å, good effects can be obtained. Below 1900 Å, absorption by quartz, water and air interferes with the experiment.

C. EFFECT OF RADIATION FROM CHEMICAL REACTIONS UPON LIVING CELLS

The same effect which has been demonstrated above as the result of irradiation with ultraviolet of known wave lengths, can be produced also by exposing the cells of microorganisms to the emanation from chemical reactions.

One of the simplest examples is the stimulation of the bacterial growth rate by the emanations from the neutralization of NaOH with HCl. WOLFF and RAS (1933b) allowed these two chemicals, flowing from two tubes, to unite on a quartz plate, underneath which was the bacterial culture. After exposure, these cultures were incubated for 2 hours. Table 13 shows very distinctly in both experiments that an exposure of approximately 5 minutes to the radiation of the neutralization process has stimulated the growth; the number of cells has been increased approximately 40-50 %.

The same authors found that even the dissolution of NaCl in water produces a growth-stimulating radiation (Table 14). This energy emission occurs only during the act of dissolving; it ceases completely when all the salt is in solution. No effect is noticeable when sugar is dissolved in water, or when palmitic acid is dissolved in alcohol. WOLFF and RAS concluded therefore that the process of dissociation of salt into ions is the source of ultraviolet.

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| | | | | _ | | | | | | | | | |
|------|-----------|-----|-----|---|-----|-----|-----|----|----|--------------------------|----------------|--|--|
| | Ex | pos | æd | 1 | for | | | | | Cells per cc. of culture | | | |
| | | | | _ | | | | | | 1 | . II | | |
| 0 mi | nutes (co | nt | rol |) | | | | | | 26 100 | 26 800 | | |
| 4 | ,, | • | | | • | | | | | 26 200 | 31 500 | | |
| 4.5 | " | · | · | | • | • | • | • | | 27 250 | | | |
| 5 | ** | • | - | • | • | ٠ | • | • | • | 37 750 | 39 500 | | |
| 6 | " | · | • | ٠ | • | ٠ | · | • | • | · | 30 900 | | |
| each | number | is | tb | e | a | 7ei | ras | ze | of | 9-11 experiments | 3_4 avanimenta | | |

Table 13. Staphylococci exposed through quartz to the energy manations of the reaction NaOH+HCl = NaCl+H₂O and counted 2 hours later

In the same way, bacterial growth was accelerated by exposure to metallic zinc in a solution of lead acetate or copper sulphate.

Simple oxidation processes also emit energy which can cause an increase in the growth rate of bacteria or yeasts. This is already cited in the method of obtaining oxidation spectra, and may be further illustrated by an unpublished experiment of Miss A. J. FERGUSON. Oxalic acid was oxidized with permanganate in a glass vessel. Above this were fastened two small covered dishes, one of quartz and one of glass, each containing a sample of the same culture of *Bacterium coli*. The sample in the quartz vessel grew more rapidly, having been exposed to ultraviolet light from the oxidation process; the other received no stimulus since glass

| Duration of | Cells per cc. of culture | | | | | | | | | | | |
|------------------------------------------------|--------------------------|--------|--------|--------|--------|--------|-------|-------|----------------|--|--|--|
| Exposure | 0 Control | 10″ | 20′′ | 30″ | 45″ | 1' | 1.5′ | 2′ | 4' | | | |
| NaCl in water | 32 100 | 31 400 | 37 700 | 45000 | | | | | <u> </u> | | | |
| | 21750 | — | | 31500 | 27 800 | | _ | | | | | |
| | 35500 | | 47500 | 45500 | | 40 000 | 36800 | | | | | |
| sucrose in water | 7 0 0 0 | | | 6250 | ! | 7250 | 7250 | 7 250 | | | | |
| palmitic acid in alcohol NaCl after com- | 7000 | - | _ | 7000 | | 8000 | 8 500 | 7 250 | 7 500 7 750 | | | |
| plete solution | 21750 | | | 22 000 | 20750 | · · | | | | | | |

Table 14. Staphylococci exposed through quartz to the energy emanation from dissolving substances

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absorbs the radiation (Table 15). There is the usual lag period of 2 hours, but after the bacteria once start to grow, the irradiated culture grows more rapidly.

Table 15. Development of a culture of Bacterium coli after exposure to emanations from the reaction

| | | | | | · | | | | | | Cells per | cc. of culture |
|------------------------------|------|-------|--|---|---|--|---|----|---|---|--------------------------|---------------------------|
| | • | | | | | | | | | | exposed through glass | exposed through quartz |
| Immediately after exposure . | | | | | | | | รณ | e | | 149 | 149 |
| 1 | hour | later | | | | | • | | | | 154 | 140 |
| 2 | | | | | | | | | | | 253 | 216 |
| 3 | | | | | | | | | | | 940 | 1735 |
| 4 | " | •• | | • | | | | | | • | 3335 | 9085 |

 $\mathrm{C_2O_4H_2} + \mathrm{KMnO_4} = \mathrm{K_2CO_3} + 2 \ \mathrm{MnO} + 9 \ \mathrm{CO_2} + 5 \ \mathrm{H_2O}$

What holds true for the simpler chemical reactions, is also correct for the more complicated biochemical processes. Proteolysis by enzymes yields an ultraviolet emanation which greatly stimulates the growth of yeast as seen in Table 16, containing the data obtained by KARPASS and LANSCHINA (1929). Of 12 experiments, only one was negative.

Table 16. Increase in the development of yeast cultures after exposure to the emanation from proteolytic processes

| Proteolytic process | Percentual increase of exposed culture over control | | | | |
|---------------------------|--------------------------------------------------------|--|--|--|--|
| Egg white with pepsin | 43 %; 20.9 % | | | | |
| egg white with pancreatin | 10.7 %; 25.8 %; 15.5 % | | | | |
| egg yolk with pepsin | 20.6 % | | | | |
| egg yolk with pancreatin | 30.1 %; 31.4 % | | | | |
| fibrin with gastric juice | 37.1 %; 36.6 %; 20.4 %;16.5 % | | | | |

All other enzymic processes which have been tested so far have yielded positive growth stimulation. Since all organisms display processes liberating energy, it is only logical to assume that all living organisms radiate. This statement must be modified somewhat by the consideration that these ultraviolet rays are

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very readily absorbed, and, for example, will not pass the skin of man or animals. Which parts of the various animals and plants radiate, will be discussed in Chapters IV and VII. Attention should be called here only to the fact that there may be radiations and growth stimulation inside of an organ or tissue without becoming noticeable outside this focus. Since we must expect ultraviolet radiations from very many biochemical processes, and since they may stimulate cell division they may play an extremely important role in the development of all living beings.