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Effects of Ultra-Violet Light on Nucleic Acid and Nucleoproteins and Other Biological Systems[1]

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It may be helpful to start with a rather general picture of the chemical and physico-chemical background of U.V. processes and also to discuss in a rather general way the possible links between the chemical phenomena and the biological effects observed. I think it will be clear that there is at present a very considerable gap between the chemistry and the biology, i.e. it is impossible to say precisely what the biological effects will be, even if we know what chemical actions were produced.

The action of light on simple chemical compounds is fairly well understood. The general result of the absorption of light by a system of nuclei and electrons is an electronic excitation, i.e. the displacement of one or more electrons from the levels they occupy in the unexcited molecule to excited states of higher energy. The energy so gained may be used in the following ways:

(1) Re-radiation (fluorescence; phosphorescence).

(2) Degradation to thermal vibrations of atoms.

(3) In bringing about chemical changes in the excited molecule, e.g. a breakage of co-valent or other bonds resulting in dissociation of the excited molecule. This happens when the energy level of the excited state is above that of the dissociated molecule.

(4) By collision with other atoms or molecules: the transfer of excitation energy to colliding molecules may occur and chemical reactions may as a consequence take place in these.

Many examples of such processes are known. In applying these concepts to biological systems two kinds of difficulties arise:

(1) Many of the absorbing molecules, e.g. proteins, nucleic acids, are extremely complex and it is usually impossible to decide what is the activated state and to trace its history.

(2) Of the many compounds which are capable of absorbing radiation, it is not known and can only be decided by indirect evidence, which are important and which are secondary in bringing about the biological effect.

[1] Lecture given to the Congrès International de Photobiologie, Amsterdam, August 1954.

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Much of the biological work has been concerned specifically with mutation. It must not be forgotten that this is a highly specialised result of u.v. radiation, since of the many actions which occur, most are lethal if the dose is sufficient and very few result in mutation. In the study of mutations produced by light we are dealing with a *non-typical* process. The lethal and other effects of light are equally important as the genetical effects. They are probably due to action of light on the *enzyme systems*. This is a little explored field which would repay examination–as many enzymes have prosthetic groups which absorb in u.v. or visible. It is probable that many of the effects of u.v. are due to disorganisation of the metabolism in the cell by inactivating or modifying critical metabolic systems.

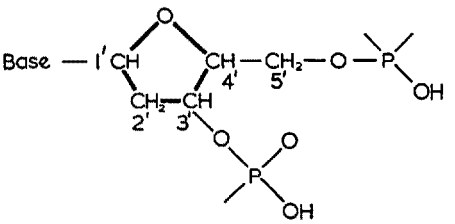

Fig. 1.–Structural formula of DNA.

It has been found that in many cases the action spectrum of light corresponds to the u.v. spectrum of nucleic acids. Since chromosomes contain DNA it is inferred that the light producing mutations is that absorbed by DNA. There are exceptions, however, e.g. the observation of MCAULEY and FORD that the maximum efficiency of irradiation of *Chaetomium* corresponds with absorption spectrum of proteins[1]. There are also the observations of HOLLAENDER of two peaks in the action spectrum[2], one of which corresponds to absorption by proteins. I shall deal here principally with the action of u.v. on desoxyribonucleic acids. The formula of a typical part of the DNA chain is shown in Figure 1.

Direct action of u.v. on nucleic acids.–Although the absorption coefficients of nucleotides and of DNA are high, the quantum efficiency of chemical changes

[1] T. M. MCAULAY and A. L. FORD, Heredity *1*, 247 (1947).

[2] A. HOLLAENDER, K. B. RAPER, and R. D. COGHILL, Amer. J. Botany *32*, 160 (1940).

produced is apparently low, but very few accurate measurements of chemical changes produced by u.v. light on these compounds have been made. ERRERA[1] gives values of the order of 10^{-2}–10^{-5} in certain cases. It has been found that purines are very stable to u.v.; pyrimidines are less so, the pyrimidine ring being decomposed with the formation of urea[2], but it is doubtful if this occurs to any appreciable extent when the pyrimidine is present in DNA. There is a loss of viscosity of DNA (Fig. 2), but it is not certain that this is the result of the breakage of nucleotide chains[3]. It is difficult to be sure of this as only one break per particle will produce a considerable decrease of viscosity; however, no great change of molecular weight has been detected as a result of u.v. radiation. It is difficult to be sure, as the molecular weight methods can not be

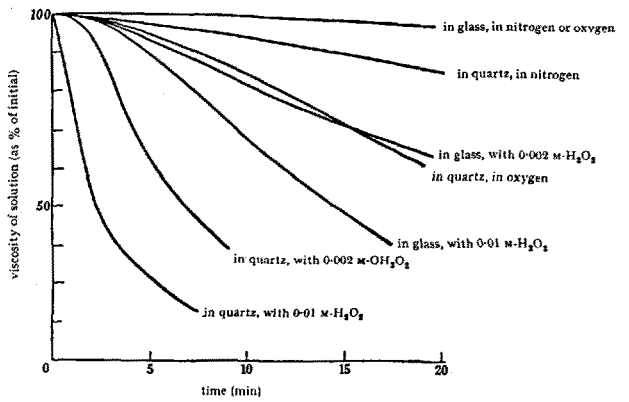

Fig. 2.–Change of viscosity of thymonucleic acid (0·1%) solutions with time when irradiated under various conditions, by means of mercury u.v. arc. at constant intensity [J. A. V. BUTLER and B. E. CONWAY, Proc. Roy. Soc. [B] *141*, 562 (1953)].

applied easily to mixtures. It must be admitted that this conclusion may have to be reconsidered when more precise methods of measurement are available. However, it is more likely that the loss of viscosity is due to a partial collapse of the stiff DNA particle due to breakage of the hydrogen bonds which maintain the original configuration. The amount of energy absorbed is considerable. Much of the energy absorbed may be degraded to vibrational energy and lost as heat. One quantum of wavelength 2500 Å = 4·96 e.v. = 115 kcals/mol, is sufficient to break at least 20 hydrogen bonds.

In case of proteins there is fairly clear evidence that u.v. radiation is capable of producing denaturation by breakage of H bonds e.g. CLARK[4] (1937–1945) found that exposure to u.v. light lowers the flocculation temperature of serum albumen.

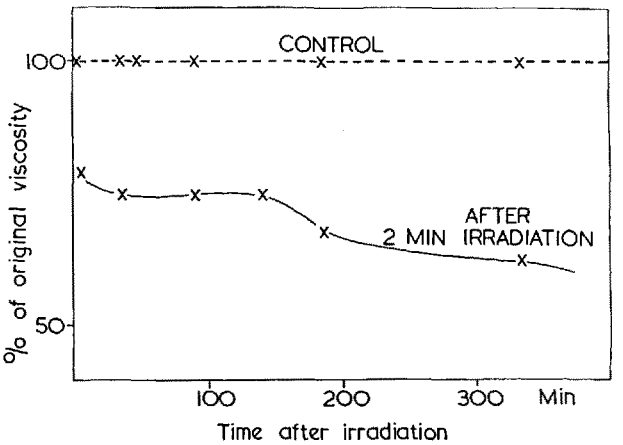

Fig. 3.–Change of viscosity of 0·05% DNA solution when kept at 40°C after 2 min u.v. irradiation in quartz tube in nitrogen.

We have found some indication that after u.v. irradiation, DNA is also more sensitive to heat degradation than originally (Fig. 3). This indicates that u.v. brings about changes which predispose the particle to heat denaturation and it is therefore probable that some hydrogen bonds are broken during the irradiation.

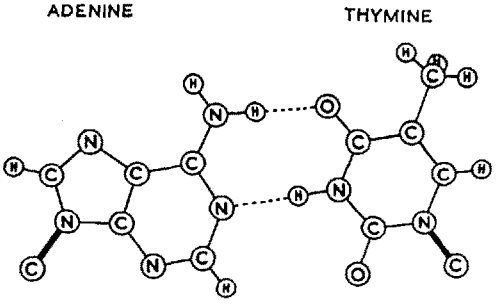

Fig. 4.–Hydrogen bonding of bases of DNA. [F. H. C. CRICK and J. D. WATSON, Nature *171*, 737 (1953).]

In the case of DNA it is possible to give a more precise interpretation of the hydrogen bond breakage in terms of the WATSON and CRICK model[1], according to which two nucleotide threads are hydrogen-bonded together (Fig. 4). It is however, not at all clear, as we shall see later, why breakage of such hydrogen bonds should produce any genetical effects as they could easily be remade, and can only be remade in one way. However, it would be very difficult to assert that u.v. does not also cause minute amounts of deamination or dehydroxylation which would have a permanent effect. I shall discuss the biological effects of such changes later.

Indirect actions of u.v. and visible light.– We turn now to cases in which light is absorbed by other molecules in the presence of nucleic acids.

[1] M. ERRERA, Biochem. Biophys. Acta *8*, 30, 115 (1952); Progress in Biophysics *3*, 88 (1952).

[2] M. M. STIMSON and T. R. LOOFBOUROW, J. Chem. Soc. *8*, 44 (1940).

[3] J. A. V. BUTLER and B. E. CONWAY, Proc. Roy. Soc. [B] *141*, 562 (1953).

[4] J. B. CLARK, Amer. J. Physiol. *113*, 538 (1935); J. Gen. Physiol. *19*, 199 (1935); *27*, 101 (1943). – E. V. RAJEWSKI, Biochem. Z. *227*, 272 (1930).

[1] J. D. WATSON and F. H. C. CRICK, Nature *171*, 737 (1953).

(1) The dissociation of oxygen itself can be brought about by wavelengths < 2530 Å. The oxygen atoms formed are, of course, powerful oxidizing agents easily producing OH radicals, which can react directly with nucleic acid. The can also give rise to hydrogen peroxide in the solution, which will have effects discussed below. It is also possible that O_2 will be activated at rather lower wavelengths by light absorbed by the nucleic acid, although this has not been proved. DNA is certainly more rapidly degraded by u.v. light (2500 Å) in the presence than in the absence of oxygen (see Fig. 1).

(2) Hydrogen peroxide when present is dissociated with practically unit efficiency by light of wavelength $<$ 3100 Å, and the main products is hydroxyl radicals: $H_2O_2 \rightarrow 2\,OH$. There are certain secondary reactions possible e.g.

$$H_2O_2 + OH = H_2O + O_2H$$

but these are probably unimportant in the presence of an oxidizable substrate. The action of OH radicals on DNA is quantitatively very similar to that of X-rays. The loss of viscosity is of the same order as that produced by a quantity of X-rays which yield an equal number of OH radicals. Among the actions of photochemically formed hydroxyl radicals, which have been observed[1] or inferred are:

(1) deamination of the bases,
(2) dehydroxylation of the bases,
(3) partial destruction of pyrimidine ring,
(4) breakage of bond between the base and the sugar,
(5) oxidation of alkyl groups (in the case of ethyl phosphates),
(6) the breakage of the nucleotide chain and the liberation of phosphate.

It might be noted that the latter seems to be a consequence of the oxidation of the sugar moiety. This has not been proved directly, but in the case of the ethyl phosphates it is found[2] that an equivalent oxidation of the ethyl group always accompanies the liberation of the free phosphate. It is also found that with DNA the phosphate liberated increases approximately as the square of the time (Fig. 5)–which is due to the fact that in order to liberate a phosphate group, *two* adjacent sugar-phosphate links have to be broken. This explains why the yield of free PO_4 from small doses of OH radicals is very small, although the efficiency of the liberation of free phosphate from alkyl phosphates and simple nucleotides by the action of OH radicals is remarkably high, about one phosphate being liberated with adenosine for each H_2O_2 decomposed. It may be that a short chain reaction is occurring. But at least we can infer from this that the hydroxyl radicals produced by irradiation of hydrogen and other peroxides are very efficient in breaking the nucleotide chain, as well as bringing about other degradative reactions. This is in strong contrast to the effect of u.v. alone.

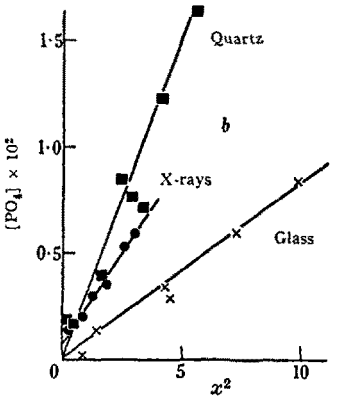

Fig. 5.–Amount of free phosphate liberated by irradiation under different conditions from thymonucleic acid, plotted against square of time. The unit of time is that which corresponds to the decomposition of 0·167% of 0·01 M-hydrogen peroxide; or in the case of the X-irradiation to 10^4 *R*. The X-ray data are from J. WEISS and M. E. SCHOLES, Exp. Cell. Res. Suppl. 2, 219 (1952). [J. A. V. BUTLER and B. E. CONWAY, Proc. Roy. Soc. [B] 141, 562 (1953).]

(3) *After-effects of irradiation of DNA.*– I might refer here to the after-effects of irradiating DNA. As mentioned above, u.v. irradiation causes some after-effect on the viscosity in the absence of oxygen. It is much more marked in presence of oxygen. In the presence of hydrogen peroxide the loss of viscosity initiated by u.v. light continues in the dark until it is practically complete (Fig. 6). It is difficult to distinguish a genuine "after-effect" from the slow-continued action of hydrogen peroxide, because although the peroxide does not act on purified DNA, it is activated by and can then degrade DNA which has been damaged by X-rays[1] and the same might possibly be true of u.v. It is also possible that DNA which has been irradiated

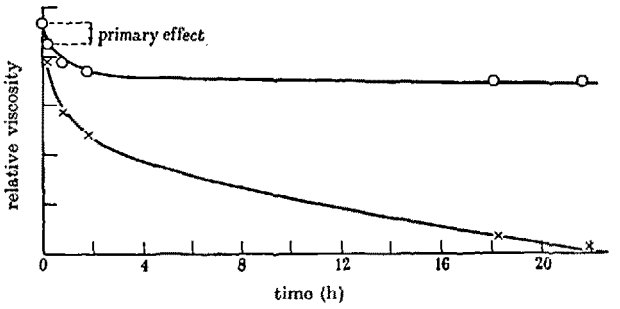

Fig. 6.–"After-effects" of irradiation of thymonucleic acid with hydrogen peroxide in quartz tube. –O– Effect of hydrogen peroxide (5×10^{-3} M). –×– Effect of ultra-violet (0·5 min) in same solution. [J. A. V. BUTLER and B. E. CONWAY, Proc. Roy. Soc. [B] *141*, 562 (1953).]

[1] J. A. V. BUTLER and B. E. CONWAY, Proc. Roy. Soc. [B] *141*, 562 (1953). – B. E. CONWAY and J. A. V. BUTLER, J. Chem. Soc. *1952*, 834.

[2] J. A. V. BUTLER and B. E. CONWAY, Proc. Roy. Soc. *141*, 562 (1953).

[1] This may not take place in the course of the enzymic decomposition of H_2O_2 by catalase. It is possibly due to imparities in the catalase.

by u.v. can initiate a radical decomposition of H_2O_2 which continues in the dark. However, the question of whether H_2O_2 can act on DNA in the dark is largely an academic one, as tissues contain substances, e.g. ascorbic acid and cysteine, which are capable of activating H_2O_2[1].

In view of the fact that catalase is sometimes added to remove H_2O_2 in experiments with viruses it might also be noted that the addition of catalase to remove hydrogen peroxide does not diminish the amount of phosphate liberated e.g. a solution of adenosine 5-phosphate, which, after illumination in the presence of H_2O_2 contained 3·9 γ per ml of free phosphate, on standing for 24 h with catalase (which reduced the concentrations of H_2O_2 to zero), had 5·4 γ; but without catalase, 4·9 γ of free phosphate. This is probably due to the formation of small amounts of radicals in the reaction between H_2O_2 and catalase[2]. Even without any illumination, catalase-H_2O_2 releases a significant amount of phosphate from adenosine-5-phosphate.

I must also mention the possibility, discussed by WEISS and SCHOLES[3], that phosphate is "labilized" by OH radicals owing to the oxidation of the O_4 carbon of the ribose, the keto-phosphate so formed being unstable and slowly hydrolysed by water. That such an effect probably occurs to some extent is shown by the following experiment with adenosine (ribose) 5-phosphate. 1 h treatment at 70° with 0·5 N H_2SO_4 hydrolyses very little phosphate from the unirradiated adenosine 5-phosphate (Table) but an appreciably greater amount after treatment with H_2O_2 + u.v.

It can be seen that the amount of "labile" phosphate formed is of the same magnitude as that liberated during the irradiation[4].

Table–Liberation of phosphate from adenosine 5-phosphate

	Free PO_4 (initial) γ per ml	Free PO_4 (After 1 h hydrolysis with 0·5 NH_2SO_4) γ per m
1. Adenosine 5-phosphate . . .	0·26	0·34
2. Same after 1 h u.v. + H_2O_2 .	3·3	6·8
3. Same after standing 24 h . .	4·4	6·5

(4) *Formation of peroxides by u.v.-light.* There is also the related subject of peroxides produced by the action of u.v. on broth, etc., by which, as WYSS and STONE have shown[1], mutations are produced, as easily as by direct exposure to u.v. These peroxides can be formed:

(a) by the action of H_2O_2, formed as described above, on organic compounds,

(b) by the direct action of oxygen atoms on organic compounds,

(c) by the reaction of the activated compound on molecular oxygen, e.g. $R \rightarrow \overset{*}{R}$; $\overset{*}{R} + O_2 \rightarrow RO_2$.

Such peroxidic substances will behave similarly to hydrogen peroxide; they can produce OH radicals by spontaneous decomposition and by reacting with activating substances such as cysteine; radicals are also produced to some extent, probably, in the action of peroxidases. Peroxides like butyl hydroperoxide in presence of Fe^{++} easily cause radical degradation of DNA[2].

(5) *Action of other absorbing molecules*, e.g. dyes. This is a very large subject which has been very little explored. Dyes like methylene blue, when activated by light, can dehydrogenate the prosthetic groups of enzymes, and as result cause marked interference with metabolic processes. More deep seated changes may also be brought about e.g. in the presence of O_2, H_2O_2 may be formed, which may react on genetic elements in the ways I have discussed above. The photodegradation of DNA by illumination in presence of dyes and carcinogen substances has been reported[3]. In general a large amount of illumination is required. In our own experiments it was found to be difficult to get consistent results owing to denaturation effects, but in some cases at least oxygen has acted as an inhibitor of degradation process, so that the primary action did not appear to be the activation of molecular oxygen.

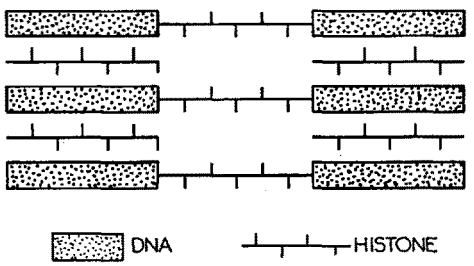

Fig. 7.–A possible structure of chromosomes: DNA particles joined by histone links.

Biological Consequences.–We can now ask, to what extent can these chemical actions, produced by u.v. and other radiations, be used to explain the biological effects e.g. chromosome breaks? The Watson and Crick model[4] provided a possible explanation of how the duplication

[1] B. E. CONWAY, Brit. J. Radiol. *27*, 42 (1954); Nature *173*, 579 (1954).

[2] B. E. CONWAY and J. A. V. BUTLER, J. Chem. Soc. *1952*, 834.

[3] J. WEISS and M. E. SCHOLES, Exp. cell Research, suppl. *2*, 219 (1952); Nature *171*, 920 (1953).

[4] For further experiments see J. WEISS and M. E. SCHOLES, Exp. cell Research, suppl. *2*, 219 (1952); Nature *171*, 920 (1953), and J. A. V. BUTLER and P. SIMSON, Liège, Symp. on Radiobiology, *1955*, 46.

[1] W. S. STONE, O. WYSS, and F. HAAS, Proc. Nat. Acad. Sci. *33*, 59 (1947).

[2] J. A. V. BUTLER and K. A. SMITH, Nature *165*, 847 (1950).

[3] G. OSTER and A. D. MCLAREN, J. Gen. Physiol. *33*, 215 (1950). H. KOFFLER and I. L. MARKEDT, Proc. Soc. Exp. Biol. Med. *76*, 90 (1951).

[4] J. D. WATSON and F. H. C. CRICK, Nature *171*, 737, 964 (1953).

of a DNA fibre with its bases in a specific order can occur. One half of the double fibre is exactly complementary to the other half, so that each half can act as a template for the other. It is obvious that if one base is damaged by loss of $-NH_2$ or $-OH$, or more drastic ways, the reproduction of the fibre at this point could not occur and this would result in a break in the newly synthesized fibre. We have seen that hydrogen and other peroxides, both with and without u.v. illumination, can bring about such reactions and also cause complete breaks in the nucleotide chain.

There is little evidence that u.v., in the absence of oxygen or peroxides, can bring about such effects, at least on a detectable scale, although it may cause a kind of denaturation of the DNA as the result of breakage of hydrogen bonds. It is, as I pointed out, difficult to see how the mere breakage of hydrogen bonds between the two halves of the double fibre could produce permanent effects. However, what happens to nucleic acid cannot be the whole story, as there are also peptides and proteins which are formed by processes at present unknown. The DNA present in the cell as a complex with basic proteins–either protamines or histones, which may have equally important functions. It must also be remembered that the chromosome is a much larger entity than the nucleic acid particle and chromosome breaks are a phenomenon belonging to a much larger order of magnitude than the separate DNA particles. In the rat chromosome there are approximately 10^5 DNA particles. If we identify these particles with the genes, they must, according to the results of genetics, be combined in a linear order. How can this occur? It is possible, of course, that the DNA particles are united directly with each other; but they undoubtedly come apart quite easily in the presence of 2M salt, and are then found to be present in solution as distinct particles. If I am permitted to speculate a little, one possibility is that they are joined by histone links. We have found[1] that in beef thymus nucleoprotein there are at least two distinct histones, which are distinguishable electrophoretically and have different compositions. One contains a larger proportion of lysine in place of the usual arginine as the principal basic amino-acid. It is possible that one of these histones provides longitudinal links between the DNA particles while the other provides lateral links either within a single particle determine the way in which the fibre is folded; or between parallel fibres (see Fig. 7)[1]. The junctions of the nucleic acid with histone, being probably made by a "salt-like" bond, will probably represent weak points at which breakage more easily occurs.

It is thus possible for the excitation, produced by the u.v. light absorbed in the nucleotide thread, to be transmitted down the column of parallel nucleotide plates, until it produces strong vibrations at the end which are able to break the bond which unites the DNA to the histone. The continuity of the chromosome would thus be broken. However, as I said above, the biological properties may depend on a higher degree of complexity than that carried by a single nucleic acid fibre. The activity of the transforming principle is greatly reduced by quantities of u.v. which have no effect on the viscosity of DNA[2], u.v. therefore causes damage which is not immediately apparent in the viscosity, although as we have seen it may become so on heating.

I have discussed briefly the kinds of chemical effects which may be expected to occur when u.v. light acts on living cells, and particularly on the chromosomes. It will be evident that much remains to be done before the circumstances in which the photochemical reactions occur are clearly defined. Still less is known about the biological consequences of chemical changes and it is probable that the most urgent task at the present time is the study of ways of distinguishing and defining the different kinds of radiation damage.

I am indebted to Mr. F. S. FEATES and Mr. JOHNS for assistance in the experiments quoted.

Résumé

L'auteur a examiné les conséquences chimiques de l'absorption de radiations ultra-violettes par les substances présentes dans les cellules vivantes (en particulier l'acide désoxyribonucléique).

Les effets observés sont dus: 1° à l'absorption directe des radiations; 2° à une action directe – ou activée photodynamiquement – sur l'oxygène présent. Les conséquences de cette dernière sont très semblables à celles produites par les rayons X.

En ce qui concerne l'absorption directe des radiations U.V., il se pourrait que la rupture des chromosomes soit due à celle des liens existant entre les chaînes de DNA et l'histone.

[1] P. F. DAVISON, D. W. F. JAMES, K. V. SHOOTER, and J. A. V. BUTLER, Biochim. Biophys. Acta *1954*.

[1] It has since been found that different DNA fractions are associated with the different histones [J. LUCY and J. A. V. BUTLER, Biochim. Biophys. Acta *16*, 431 (1955)]. The general picture of DNA-histone interactions given here remains possible.

[2] S. ZAMENHOF, H. E. ALEXANDER, and G. LEIDY, J. Exp. Med. *98*, 373 (1953).