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PHOTOCHEMISTRY OF NUCLEIC ACIDS, NUCLEIC ACID DERIVATIVES AND RELATED COMPOUNDS

Contents

P O S T Ę P Y B I O C H E M I I
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SUPPLEMENT

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Photochemistry of nucleic acids, nucleic acid derivatives and related compounds

It is the purpose of the present review to summarize the available facts regarding the effects of ultraviolet light on nucleic acid derivatives and related compounds, as well as on nucleic acids themselves. While one of the ultimate objectives of such information is to gain a better understanding of the way in which the biological effects of irradiation manifest themselves through the nucleic acids of living cells and to what extent the latter are involved, we shall confine ourselves largely to the physical chemistry of the processes involved, drawing on biological phenomena (particularly in the case of viruses) to some extent for purposes of illustration.

The photochemistry of nucleic acids is of considerable interest because of their important role in a variety of cellular functions. Of all cell components they are the ones which exhibit the highest absorption in the ultraviolet region where irradiation provokes numerous biological effects; and action spectra provide substantial evidence for the nucleoprotein character of the immediate receptors of such radiation. As will be shown there is even some evidence for involvement of free, as well as DPN-bound, nucleotides.*

Recent observations on the reversible photolysis of pyrimidine nucleotides has lent added interest to the photochemistry of nucleic acids in view of its possible relation to the phenomenon of photoreactivation. Although the reversibility of the photolysis of uridylic and cytidylic acids was first reported in 1949, the same year in which the phenomenon of photoreactivation by visible light was first announced, it was not until 5 years later that the former began to receive additional at-

* The following abbreviations are used here: RNA, ribonucleic acid; DNA, desoxyribonucleic acid; APA, apurinic acid; DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide; ATP, adenosinetriphosphate; TMV, tobacco mosaic virus; Ø, quantum yield.

tention. Consequently up to only 3 years ago studies on the photolysis of nucleic acids and their components concerned themselves only with the permanent degradative effects resulting from irradiation. It is to be expected, however, that increasing attention will now be devoted to the phenomenon of reversibility and particularly with regard to its biological implications.

A good deal of the earlier data on the photochemistry of nucleic acids and their derivatives may be found in the compilation of Ellis, Wells & Heyroth (1941). More recent reviews dealing with the photochemistry of nucleic acids are those of Loofbourow (1948), Giese (1950, 1953), Errera (1952c, 1953), Butler (1955), McLaren (1957).

Experimental techniques

It is astonishing how many publications deal with the effects of ultraviolet light on nucleic acids or their derivatives without presenting adequate data regarding irradiation conditions. In many instances nothing more has been stated than the type of lamp used and the distance from the sample to the lamp. It is consequently difficult to compare results from different laboratories and many of the earlier observations are for these reasons of little more than qualitative value.

Frequently the intensities or times of exposure have been considerably higher than those used for *in vivo* studies. Even 1,3-dimethyl uracil, for which the effects of irradiation may be subsequently reversed in the "dark" with high degree of efficiency, will be decomposed to a variety of photoproducts if irradiated long enough. But it is to be doubted whether the results of intensive irradiation are, in this case, of any real biological significance by comparision with the reversible photolysis occurring with lower intensities.

Wherever possible it is obviously most desirable to use a monochromator; however such instruments with efficiencies suitable even for low intensity irradiation in the ultraviolet are not widely available. The next most suitable source, by far preferable to the wide variety of high-pressure mercury lamps commonly used, is the low-pressure mercury resonance lamp with high emission at 2537 Å. Such lamps for photochemical work are available from a number of firms who usually supply data regarding emission intensities at various wavelengths, and which may attain well over 90% of the total at 2537 A. Peel (1939) reports that British Thermal Syndicate resonance lamps emit 97% of the total intensity at 2537 Å, 2.7% above 3 000 Å and 0.3% at various wavelengths below 3 000, excluding the 2537 line.

Even with such sources it is best to use some type of cutoff filter (such as diluted acetic acid) to eliminate wave-lengths below 2400 Å and especially the mercury resonance line at 1849 Å which, even though present only in trace amounts and strongly absorbed by air (50% absorption in about 2-3 cms. air), may very markedly affect the course of a photochemical reaction. Santer (1957) has recently presented data on the amount of 1849 Å irradiation emitted by resonance lamps, as well as a convenient method for measuring the intensity of the 1849 Å line.

Very suitable sources which have come into widespread use during the past few years are germicidal lamps, which are really resonance lamps in which the quartz envelope has been substituted by a thin ultraviolet transmitting glass. The characteristics of such lamps have been so well standarized by many firms that for many purposes the doses delivered to an irradiated sample may be calculated from the manufacturer's data. A table giving full characteristics of a number of such lamps may be found in an article by Buttolph (1955). Surprisingly enough even such sources may emit traces of 1849 Å (Buttolph, 1955; Canzanelli, Guild & Rapport, 1951) and suitable precautions should be adopted in such cases.

Where reasonable estimates of quantum yield are to be obtained, it is much more convenient to measure dosages by means of chemical actinometry. This is not only simpler than physical methods, but also less dependant on the geometry of the irradiation set-up used. In addition to the well-known uranyl oxalate actinometer (Forbes & Leighton, 1930; see Bowen, 1946) several new systems have been recently described for precision actinometry at very low light intensities, e. g. using malachite green leucocyanide (Calvert & Rochen, 1952). A system several hundred times more sensitive than the ordinary uranyl oxalate actinometer is that of Parker (1953) using ferrioxalate with phenanthroline. A recent improvement on the uranyl oxalate system, with very high sensitivity is that of Pitts, Margerum, Taylor & Brim (1955); this procedure has the additional advantage that determination of oxalate is made indirectly by spectrophotometric measurements. An excellent outline of the principles of chemical actinometry may be found in an article by Masson, Boekelheide & Noyes (1956). For many purposes one of a number of pyrimidine nucleotides may be used for measuring light intensities by merely measuring the changes in absorption spectrum produced.

It is beyond the scope of this article to discuss in detail the principles of photochemical practise for which reference should be made to

standard texts such as Bowen (1946) or to recent articles such as that of Scott & Sinsheimer (1955) and, particularly, Masson et al. (1956). It is, however, perhaps not out of place to state that if investigators were to give sufficient data to make possible the calculation of the amount of light absorbed by the irradiated system, comparisons with the results of other observers would be considerably facilitated. The use of thin layers of living cells by Errera (1952c) is a good illustration of the type of procedure useful in biological irradiation experiments.

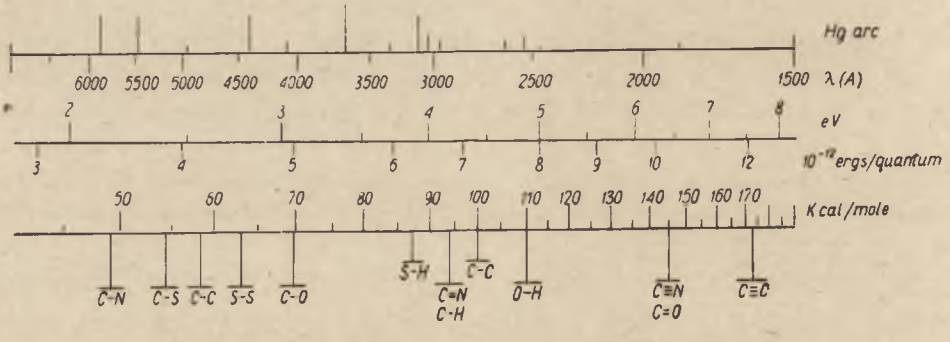


Fig. 1. Energy conversion diagram and bond energies

Because of the use of different units by various investigators to describe irradiation conditions, the attached energy conversion diagram (Fig. 1), partially adapted from Bowen (1946) may be of some assistance during the reading of this text. The bond energies in this diagram have been taken from Pauling (1948).

Purines, purine nucleosides, purine nucleotides

The single most important fact regarding purines and their nucleosides and nucleotides, on which all observers are agreed, is their relative resistance to irradiation as compared to pyrimidines (Sinsheimer & Hastings, 1949; Carter, 1950; Errera 1952a; Christensen & Giese, 1954).

As early as 1934 Runnstrom, Lennerstrand & Borei (1934) observed that, although the co-enzyme activity of DPN was destroyed by ultraviolet irradiation, cophosphorylase activity was retained intact, indicating that the adenylic acid moiety of the molecule was unaffected. If a solution of DNA is irradiated to the point where 30% of the pyrimidine bases are destroyed, the entire guanine and more than 90% of the adenine may be recovered from a hydrolyzate (Errera, 1952a).

Unfortunately quantitative data for these compounds are practically non-existent so that comparisons of the results of different observers are rendered somewhat difficult. Numerous workers have made qualitative observations on the destruction of the naturally occuring purines by irradiation, the usual criterion being destruction of the characteristic absorption spectrum between 2,000 and 3,000 Å (Heyroth & Loofbourow, 1931; Rapport & Canzanelli, 1950; Canzanelli, Guild & Rapport, 1951).

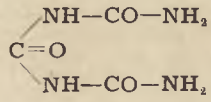
Irradiation for 12 hours with a General Electric germicidal lamp of 5×10^{-5} M solutions of adenine, adenosine, xanthine, hypoxanthine, uric acid, guanine and caffeine in all cases suffices to completely disrupt the absorption spectrum (Rapport & Canzanelli, 1950). Isoguanine also loses its characteristic absorption under the influence of irradiation (Stimson, 1942). By contrast Christensen & Giese (1954) report that prolonged irradiation of adenine, adenosine and adenylic acid leads to an increase in absorption over the range 2300—2900 Å, but no confirmation of this has been forthcoming.

For purposes of comparison with pyrimidines in this review, we have made tentative measurements of the quantum yields for adenine and guanine at 2537 Å, as measured by decrease in principal absorption maximum. The values we obtained for adenine and guanine are 0.6×10^{-4} and 2×10^{-4} respectively. No increase in absorption was noted during irradiation such as reported by Christensen & Giese.

An examination of the qualitative results of Canzanelli et al. (1951) suggests that the quantum yields for xanthine, xanthosine and hypoxanthine are somewhat higher and that these are the more sensitive to irradiation of the normally occurring purine derivatives.

The liberation of urea and ammonia by irradiated purines has been shown by Canzanelli et al. (1951) to be largely dependant on the presence of a carbonyl group in the 2-position, as is the case for pyrimidines (see p. 256 for details).

Extensive irradiation of uric acid leads to the formation of a variety of photoproducts. Fellig (1953) irradiated 2 litres of a neutral solution of uric acid, 0.1 mg./ml., with a bank of 7 General Electric germicidal lamps. Following concentration of the solution, about 10% of the initial substance was recovered in crystalline form and identified as triuret:



The irradiation of ATP is accompanied by the liberation of inorganic phosphate, adenosine-5'-phosphate and adenine, testifying to the labilization of the nucleosidic linkage by the pyrophosphate group. Adenosine and adenylic acid under similar conditions are unaffected. It should be pointed out that the intensities of irradiation in these experiments was „too high to measure directly with conventional instruments". (Carter, 1950). The same author found that the decomposition of DPN is accompanied by the rupture of nucleoside and nucleotide linkages.

More extensive studies of irradiation of DPN and TPN have been made by Seraydarian, Cohen & Sable (1954) and Seraydarian (1955). Following a period just sufficient to destroy DPN activity, the following four products were separated by ion-exchange: adenosine diphosphate, and lesser quantities of adenylic acid, adenine and nicotinamide. Some of the latter product was destroyed, as was half the ribose. Since no adenosine was found in the irradiation products, in agreement with Carter (1950), despite the presence of adenylic acid and adenine, it was concluded that the ribosidic linkage is more susceptible to irradiation in the nucleoside than in the nucleotide form. In agreement with the above results only a small decrease in ultraviolet absorption was recorded. Irradiation of TPN, to the point where only 10% of the coenzyme activity is retained, is accompanied by only a 2-3% decrease in optical density, a 30% decrease in ribose and appearance of 15% of the total P as 7-minute hydrolyzable phosphate, with the appearance of the following products (separated by ion exchange): nicotinamide and some complex of nicotinamide with unidentified products of ribose; adenosine - 2', 5'diphosphate and 2' (or 3')-phospho-adenosine diphosphate. Both DPN and TPN therefore behave similarly, as might be expected; for both there is a loss of ribose of the nicotinamide moiety, some splitting of the ribose phosphate-ester of the nicotinamide nucleotide moiety leaving the pyrophosphate group intact and attached to adenosine in the case of DPN and adenosine-2'-phosphate in the case of TPN (see also p. 289).

Seraydarian (1956) has recently examined qualitatively the effect of wavelength on the decomposition of DPN and TPN, showing that complete inactivation may be achieved without using wavelengths below 2100 Å.

Two groups of observers have reported some type of „reactivation" of irradiated ATP. Kita, Maeda, Hanazaki, Shimizu & Fujita (1954) state that the decrease in absorption of ATP, resulting from irradiation with an undefined ultraviolet lapm, may subsequently be

reversed under the influence of visible light. Garay & Guba (1954), using a high-pressure Hannau Lamp without filter, report an increase in absorption of ATP upon irradiation and that this is due to a change in configuration as a result of interaction with free radicals formed from the irradiated aqueous medium; with time the photoproduct is said to revert to the original ATP. The same effect is reported to occur with ultrasonic irradiation. Not only are these results in contradiction with those of other observers; we have carefully examined the above publications and find that the texts in both cases do not appear to substantiate the conclusions drawn.

Pyrimidines & pyrimidine nucleosides and nucleotides

A good deal more is known about the photochemistry of this class of compounds than is the case for purine derivatives. One may divide pyrimidine derivatives into two groups from the point of view of photochemical behaviour: (a) uracil and cytosine, as well as a number of their derivatives including nucleosides and nucleotides as well as some N- and O-alkyl derivatives, the photolysis of which is to a greater or lesser degree reversible and which is accompanied by the uptake of a water molecule by the 5 : 6 double bond in the pyrimidine ring; (b) derivatives substituted in the 5 or 6 positions, e.g. thymine, 5-methylcytosine, orotic acid, the irradiation of which leads to the destruction of the pyrimidine ring with the formation of a variety of products. In addition to these two groups there are a number of other derivatives which have thus far received little or no attention, but for most of which the reaction is probably not reversible, e.g. thiocytosines, thiouracils, etc.

Up to 1949 the opinion was fairly general that irradiation of all pyrimidines leads to destruction of the aromatic ring. This was undoubtedly due in large part to the indiscriminate use of intense irradiation sources, frequently with no attempts to eliminate wavelengths much shorter than 2537 Å so that what was frequently observed was the destruction of the primary photoproducts in the case of such compounds as uracil.

The photolytic degradation of thymine was studied by Bass (1924) who irradiated this compound in the presence of ferrous salts and oxygen and concluded that the reaction involved rupture of the pyrimidine ring, since he was able to show the presence of urea and pyruvic acid after several hours irradiation. Johnson, Baudisch & Hoffman (1932) investigated the behaviour of glycolthymine in acid and neutral medium

under the influence of daylight or carbon arc radiation. In acid medium they observed the formation of a new anhydride of glycolthymine which was accompanied by decolorization of the solution and the appearance of a green fluorescence under the influence of ultraviolet irradiation. In neutral medium destruction of the pyrimidine ring occurs with the formation of acetol. Both these studies are actually of more interest from a historical point of view since they do not really deal with the direct effects of irradiation.

The photochemical behaviour of uracil was first investigated by Heyroth & Loofbourow (1931) who followed the change in absorption spectrum resulting from the exposure of this compound to a water-cooled Victor mercury arc and found that the principal absorption maximum gradually decreased to zero with a simultaneous increase in absorption at 2200—2300 Å and above 2900 A . Subsequently Heyroth (1932), seeking an explanation for the appearance in irradiated blood of substances capable of reducing arsenophosphotungstic acid, observed that the irradiation of uracil resulted in the formation of a substance (or substances) also capable of reducing the above-mentioned acid, particularly during the initial stages of irradiation.

Discussing the possible mechanisms of photolysis of uracil, Heyroth & Loofbourow (1941) advanced the hypothesis that the initial stage of this reaction could be due to the formation of photoproducts such as 2,5-dioxypyrimidine (isouracil) since, according to Johnson & Johns (1914) only pyrimidines possessing an amino or hydroxyl group in position 5 of the ring are able to reduce alkaline solutions of phosphotungstic acid. It is curious that this observation of Heyroth's has gone unnoticed, since it may have some relation to the primary photoproduct of uracil which may revert again to uracil in the "dark"under suitable conditions. It should be noted in this connection that the presence of a hydroxyl or amino group in position 5 of the pyrimidine ring is not a sufficient condition for the ability of the latter to reduce alkaline phosphotungstate (or molybdate). An additional hydroxyl or amino group must also be present at 6 or 4 (Bendich & Clements, 1953).

The first attempt to quantitatively follow the course of photolysis of a pyrimidine is that of Uber & Verbrugge (1940) on the pyrimidine component of thiamine, viz. 2-methyl-5-ethoxymethyl-4-aminopyrimidine, at pH 4.8, using a Hanovia resonance lamp. The course of the reaction was followed spectrographically and the quantum yield was calculated from the loss in biological activity, the spectral measurements being used to correct for the decrease in absorption of the solution during the course of irradiation. The reaction was found to proceed with a quantum yield of 1.84×10^{-2} and was accompanied by a decrease

of the entire absorption spectrum without any change of shape. From ammonia determinations the authors concluded that 1/3 of the amino nitrogen was released by photolysis. In view of the fact that, at any given stage during irradiation, the optical density of the solution was always higher than what it should have been according to the biological quantum yield, the suggestion was advanced that the reaction mechanism involves the removal of the amino and, perhaps, other side groups either simultaneously with or preceding the rupture of the pyrimidine ring. However, removal of side groups as the initial reaction resulting from irradiation does not appear to us as very plausible since such a process should be accompanied by a shift in the absorption spectrum; no such shift is observable in the spectra presented by the authors for various periods of irradiation. Furthermore the appearance of ammonia in the photolysed solution is by itself no evidence of the removal of the amino group since, as we shall see below, ammonia frequently appears as a photolysis product of the pyrimidine ring.

Stimson & Loofbourow (1941) observed that 2-chloro-6-aminopyrimidine is as resistant to irradiation as adenine, concluding from this that the susceptibility to photolysis parallels the number of carbonyl groups in the ring. This assumption was apparently based on a qualitative comparison of the behaviour of this compound with that of uracil (Heyroth & Loofbourow, 1931) and barbituric acid (Loofbourow & Stimson, 1940) under similar experimental conditions.

It is, however, not strictly true. Canzanelli, Guild & Rapport (1951) have shown that it is rather the presence of a carbonyl group in the 2-position of the ring that influences the susceptibility to irradiation.

In 1949 Sinsheimer & Hastings reported that the photolysis of uracil, uridine and cytidylic acid could be reversed by subsequent acidification to pH 1, the criterion for reversal being the re-appearance of the original absorption spectrum of the irradiated substance. They pointed out that the course of the reaction was to a considerable extent dependant on the nature of the irradiation source and, in particular on the presence or absence of wave-lengths below 2300 Å. (such as the Hg lines at 1849, 1942 and 2242 Å). Reversibility could be observed only in the absence of these lines from the source; and the presence of these shorter wavelengths leads to ring rupture in the primary reaction with a considerably higher quantum yield than for longer wave-lengths.

Despite the obvious importance of these findings in a number of biological problems, they went almost unnoticed for five years until Sinsheimer himself took up the question again.

The photochemical transformation of uracil (Sinsheimer & Hastings, 1949, Sinsheimer, 1954) in neutral medium (0.1M phosphate buffer pH 7) proceeds with a quantum yield of 0.052 and is accompanied by the disappearance of the characteristic absorption maximum; under these conditions about one-half the uracil is transformed to a product which reverts to uracil upon acidification (pH 1) or heating. The remainder of the uracil is destroyed with the formation of a variety of unknown photoproducts.

A qualitatively similar behaviour is exhibited by 1-methyluracil (Wierzchowski & Shugar, 1956, 1957) and 1,3-dimethyluracil (Moore & Thomson, 1955, 1956, 1957; Wang, Apicella & Stone, 1956). The latter authors were succesful in completely eluclidating the mechanism of the reaction as well as the structure of the photoproduct, since the physico-chemical properties of dimethyluracil made possible the isolation of the photoproduct in a pure state (Moore & Thomson, 1957).

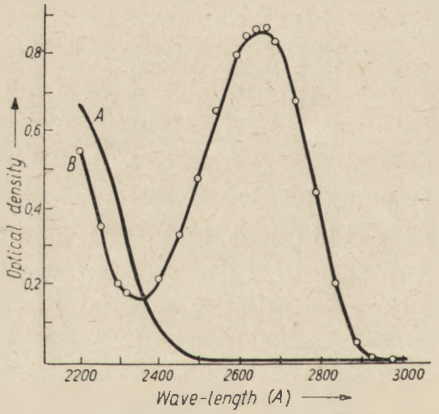


Fig. 2. Photolysis, at 2537 A, and subsequent reversibility, of 1,3-dimethyluracil (10^{-4} M in H_2O):Curve B, 1,3-dimethyluracil in water; Curve A, product of irradiation; points on curve B, product of irradiation after heating 5 mins. at 100°C at pH 1 (Moore & Thomson, 1955)

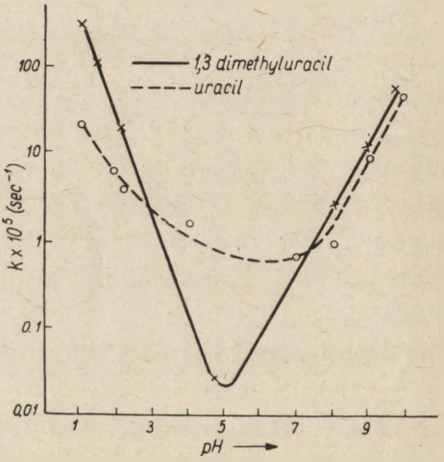


Fig. 3. Variation with pH of the first-order rate constant K (at 20°C) for the regenaration of uracil (– – – –) and 1,3-dimethyluracil (———) from their respective photoproducts (Moore & Thomson, 1956)

Figs 2 & 3 (Moore & Thomson, 1955, 1956) illustrate the course of the photochemical reaction for 1,3-dimethyluracil as well as the pH dependance of the subsequent reverse acid-base catalyzed reaction for uracil and dimethyluracil. The activation energy for the reverse reaction in acid medium was found to be 22.6 kcal./mole for dimethyluracil.

Elementary analysis of the photoproduct, and its molecular weight of 158.3 by comparision to that of 140 for dimethyluracil, indicated uptake of a water molecule by the pyrimidine ring during irradiation. That the point of attachment is the 5: 6 double bond is shown not only by the disappearance of the characteristic absorption maximum (see Fig. 2) but also by the absence of the characteristic reaction of unsaturated pyrimidine rings with bromine water. The infrared absorption spectrum of the photoproduct in alcohol-free chloroform exhibits also an intense band at 2.98 μ, corresponding to OH frequencies, which is absent in dimethyluracil. On the basis of these facts Moore & Thomson (1955) proposed that the photoproduct is either 5- or 6-hydroxy-1,3-dimethylhydrouracil. Since the properties of the known compound 5-hydroxyhydrouracil did not correspond with the properties of the photoproduct, it was concluded that this latter should be 6-hydroxy--1,3-dimethylhydrouracil.

This suggestion was confirmed by Wang et al. (1956) by synthesis of 6-hydroxy-1,3-dimethylhydrouracil which they showed to have the same elementary analysis and infra-red absorption spectrum as the photoproduct, and to revert to dimethyluracil under the influence of acid-base catalysis.

Simultaneously Moore & Thomson (1957) succeeded in synthesizing this compound as well, by a different method and independently of Wang et al. (1956); its identity with the photoproduct of dimethyluracil was established by its ultraviolet absorption spectrum, X-ray diffraction pattern, melting point and molecular weight.

Studies on uridine and uridylic acids **a** and **b** (Sinsheimer, 1954), cyclic uridine phosphate 2' : 3' (Shugar & Wierzchowski, 1957b), uridine di- and triphosphates as well as uridine diphosphate glucose (Zill, 1957) all show that substitution of the N_1 position in the uracil ring by carbohydrates, as well as esterification of the sugars, does not qualitatively affect the nature of the reaction; but does influence the magnitude of the quantum yield as does substitution of a methyl group, but to a lesser extent (Table 1). Esterification of the sugar moiety, however, as well as the position of esterification, markedly influence the stability of the photoproduct. Sinsheimer (1954) states that the rate of the reverse reaction for uridine is greater than for uridylic acids while for uridylic acid **b** at 85°C it is greater than for **a**. Shugar & Wierzchowski (1957b) report that for cyclic uridine phosphate the reverse reaction is faster than for uridylic acid **b**. The pH at which the photoproduct of uridylic acid **b** is most stable (i.e. at which the rate of the reverse reaction is at a minimum) is at 5.2 (Sinsheimer, 1954), roughly the same as that for the photoproduct of dimethyluracil (Fig. 3).

Additional evidence regarding reversion of the photoproduct to the original compound is furnished by Mitchell (1955) and Rapport, Canzanelli & Sossen (1955) who showed that the growth requirements of a pyrimidine-requiring mutant of *Neurospora crassa* could not be satisfied by irradiated uridine, but could be if the irradiated uridine were either acidified, heated, or made slightly alkaline.

Excluding for the moment compounds substituted in the 5 or 6 positions, not all uracil derivatives behave photochemically like the above compounds. In the case of 2-ethoxyuracil from neutral to alkaline pH (13) a new compound is formed, with a quantum yield of 8×10^{-3}, which exhibits 2 maxima at neutral pH, one at 2740 (with an extinction twice that for 2-ethoxyuracil) and another at 2270 Å; neither heating nor acidification results in reversion to the original compound but only in destruction of the photoproduct, A study of this reaction in light and heavy water indicate indirectly (see below) that here as well the reaction involves uptake of a water molecule, but the reaction is much more complicated than in the case of uracil (Wierzchowski & Shugar, work in progress). For O^2 : 2'-cyclouridine a new maximum at 2325 Å appears on irradiation in neutral medium; but the reaction is only partially reversible on heating (work in progress).

Table I lists a number of compounds which have been examined as regards reversibility. The second column of this table gives the quantum yields, where known. The third column is taken from Moore & Thomson (1956) and gives the relative first-order rate constants for a number of derivatives irradiated under identical conditions. One serious discrepancy exists between columns 2 and 3; the relative values of k for thymine, thymidine and thymidylic acid are not agreement with the corresponding values of Φ, but the reason for this is not clear. The reaction mechanism for those substances for which there is no reversibility has not received much attention, but undoubtedly involves ring rupture. An examination of thymine in light and heavy water has shown that the uptake of a water molecule is not involved (Table III).

On the other hand reversibility is not only dependant on the 5 and 6 positions in the pyrimidine ring being unsubstituted.*

The distribution of electron density in the pyrimidine ring is also important in this respect, as shown by the non-reversible photolysis of 2-thiouracil, (Wierzchowski & Shugar, 1957), 2-thiocytosine, 4-ethoxy-2-pyrimidone, 2,4-diethoxypyrimidine (Wierzchowski & Shugar, work in progress).

* Moore & Thomson (1956) report "very slight' reversibility for 6-methyluracil.

Table I

Quantum yields (or relative first-order rate constants) for some pyrimidine derivatives, and nature of reaction (reversible or not)

Compound	Ø (x 10^3)	k (min^{-1})	Reversibility
Uracil	5.2_a;6.0_b	0.091	+
1-Methyluracil	12.5_b	—	+
1,3-Dimethyluracil	10.4_c	0.384	+
Uridine	$21.6_{a,b}$	0.578	+
Uridine phosphate 2′	21.6_a		+
Uridine phosphate 3′	21.6_a		+
Uridine phosphate 2′ : 3′	21.6_b′		+
Uridine diphosphate	—	—	+(d)
Uridine triphosphate	—	—	+(d)
Uridine diphosphate glucose	—	—	+(d)
2-ethoxyuracil	8.0_b′	—	—
4-ethoxyuracil	very low	—	—
2,4-diethoxypyrimidine	very low	—	—
Thymine	0.4_b	0.0013	—
Thymidine	0.65_b′	0.0011	—
Thymidylic acid	1.0_b′	0.0127	—
5-Bromouracil	—	0.058	—
5-Nitrouracil	—	0.038	—
5-Bromo-1,3-dimethyluracil	—	0.384	—
6-Methyluracil	0.83_b′	0.0082	very slight (c)
Orotic acid (Na salt)	—	0.041	—
Uracil-5-acetic acid (Na salt)	—	0.0085	—
2-Thiouracil	1.7_b′	—	—
6-Propylthiouracil	—	0.021	—
Barbituric acid (pH 5.2)	—	0.059	—
1,3-Dimethylbarbituric acid	4.65_b′	—	—
1,3-Dimethyl-6-methoxybarbituric acid	4.1_b′	—	—

All compounds irradiated at concentrations of the order 10^{-3} — 10^{-1} M in neutral solution (water or buffered):

a — data given by Sinsheimer (1954)

b — „ „ „ Wierzchowski & Shugar (1957) and b′ (unpublished)

c — „ „ „ Moore & Thomson (1956)

d — „ „ „ Zill (1957)

k — first order rate constant for photodecomposition (Moore & Thomson, 1956)

The reversible reaction may also be accompanied by side reactions leading to ring rupture, e.g. in the case of uracil cited above (Sinsheimer & Hastings, 1949; Sinsheimer, 1954) only about half the product is reversibly photolysed, the remainder being decomposed to a variety of products. Moore & Thomson (1956) found that intense irradiation of a 0.2M solution of dimethyluracil resulted in the formation

not only of 6-hydroxy-1,3,-dimethylhydrouracil but also a series of coloured degradation products which could be partially separated on an Al_2O_3 column; one of these degradation products could be found also in photolysed uracil and uridylic acid.

Rapport et al. (1955) obtained 100% reversibility in the case of uridine for short irradiation periods, but report that long exposure times results in the recovery during the reverse reaction of a mixture of uracil and uridine, and finally uracil alone.

There is, however, general agreement amongst various authors that if irradiation is conducted only long enough to cause disappearance of the absorption spectrum of the pyrimidine ring, that 90% or greater reversibility may be subsequently obtained.

Conrad (1954) irradiated 10^{-3}M solutions of uracil with a GE 15-watt germicidal lamp 1 cm. from the surface of the solution, following which the solution was evaporated to dryness under vacuum. From carbon and nitrogen determinations of the photoproducts thus obtained it was deduced that 25% of the N and up to 75% of the C were lost in the form of volatile products either during irradiation or during evaporation. From the remaining material it was possible to obtain crystalline oxamide and parabanic acid as well as four other unidentifiable products in relatively pure form.

Canzanelli, Guild & Rapport (1951) identified urea and ammonia amongst the products of irradiation of 5×10^{-5}M solutions of uracil with a germicidal lamp, and suggested that the urea originates from the ureido group of the pyrimidine ring. Their results showed derivatives with a carbonyl group at position 2 give more urea and less ammonia. The ammonia would also originate from the ureido group and not from the urea formed as a result of irradiation. Of some interest is the fact that the presence of an amino group did not result in an increased production of ammonia.

Because of structural considerations neither oxamide nor parabanic acid can be formed directly from irradiated uracil as a result of ring rupture, but probably arise as a result of secondary reactions of the excited molecules or free radicals formed during the primary reaction. The general appearance of urea amongst the products does throw a little light on the primary reaction; this product could arise from the simultaneous rupture of two —C—N—bonds in the pyrimidine ring as a result of dissociation or predissociation of the excited molecule. Energetically this is possible since the —C—N— bond energy is about 56 kcal./mole as compared to 110 kcal. for 2537 Å. Where the shorter wavelengths are not filtered out, the probability of this type of primary reaction will obviously be even greater. Naturally other mechanisms are also possible

if we bear in mind the symmetry of the molecule and the influence of the symmetry of a given excited state on its dissociation into several fragments. An examination of the spectra of some pyrimidines in the vapour phase would undoubtedly assist in clarifying the mechanism of the primary reaction.

Reversible photolysis is undoubtedly the result of excitation of the pyrimidine ring leading to an altered distribution of electron density facilitating the uptake of a water molecule (Shugar & Wierzchowski, 1957a; see below). Incomplete reversibility resulting from excessively long exposure times may be the result of several factors, including slow pyrimidine ring rupture by traces of radiation below 2537 Å with unfiltered sources, destruction of the reversible photoproduct as a result of its absorption below 2400 Å with unfiltered sources, as well as indirectly as a result of radicals formed in the above-mentioned reactions.

A second group of compounds undergoing reversible photolysis at 2537 Å includes cytosine, its nucleosides and nucleotides as well as several other derivatives. A similar reaction has likewise been observed for isocytosine and its nucleoside (arabinoside).

Table II

Quantum yields (x 10^3) for photolysis of cytosine derivatives at 2537 Å

Compound	pH						
	1	2	7.1	10.5	11	13	14
Cytosine	—	1.3	1.3	—	—	6.3	8.0
1-Methylcytosine	—	1.4	1.3	—	—	2.1	—
Cytidine	—	1.6	10.0	8.9	6.8	3.6	2.9
Cytidine phosphate 2′	—	—	14.1	12.1	7.2	—	—
Cytidine phosphate 3′	—	—	9.5	9.6	6.6	—	—
Cytidine phosphate 2′+3′	1.1	—	12.5	9.4	6.3	—	—
cytidine phosphate 2′ : 3′	—	—	11.5	—	—	—	—
Desoxycytidine	—	1.6	8.6	8.3	6.0	—	—
Desoxycytidine phosphate 5′	—	1.5	2.9	3.0	3.0	1.0	—
Cytidine phosphate 5′	—	1.6	5.2	5.4	5.4	—	—
Pyranosyl-cytosines	1.6	—	13.0	11.0	7.9	1.0	0.25

Table II presents the quantum yields as a function of pH for those compounds of this group hitherto investigated. (Wierzchowski & Shugar, 1956, 1957; Shugar & Wierzchowski, 1957b).

As in the case of uracil and its derivatives the irradiation of all these compounds in neutral medium is characterized by disappearance of the characteristic absorption maximum at 2700 Å; however, the nucleosides and nucleotides all exhibit a new maximum at about 2360 Å.

(Figs. 4 & 5). Moore & Thomson (1956) report that cytidylic acid and cytosine desoxyriboside lose the ability to bind bromine at the 5 : 6 double bond during the course of irradiation.

The photoproducts are considerably less stable than those of uracil and its derivatives and exhibit the same dependance of stability with respect to pH with maximum stability at about neutrality. The activation energy for the reverse reaction is quite high but there are as yet no detailed data on the kinetics of the reverse reaction and its dependance on pH. The reverse reaction has been shown by Sinsheimer (1957) to be 1st-order for cytidylic acids **a** and **b** as it is likewise for all cytidylic acid isomers (Shugar & Wierzchowski, 1957b).

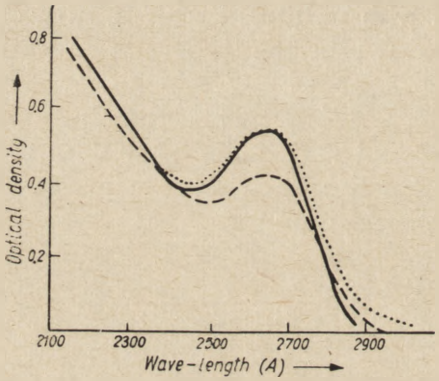


Fig. 4. Cytosine at pH 7.2,non-irradiated (———), after 85 min irradiation (— — — —), and after heating irradiated solution 10 min at 80°C (.....)

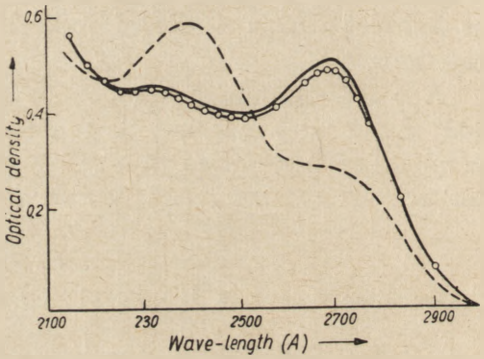


Fig. 5. 2':3'-cytidine phosphate at pH 7.2,non-irradiated (———), after 80 min irradiation (— — —), and after heating irradiated solution 5 min at 80°C (o-o-o)

At pH 7 and 30°C the half-times for the reverse reaction for cytidylic acids 2' and 3' are respectively 22 and 96 mins. (Sinsheimer, 1957), at pH 4 these values are decreased to about one-eighth and at pH 13 are too short to measure. The half-time decreases about 50-fold in going from 0° to 50°C. The reverse reaction for all cytosine nucleotides is more rapid than for uridine nucleotides (Shugar & Wierzchowski, 1957b) while cytidine-3'-phosphate is more stable than the 2' isomer in contrast to the uridylic acid isomers where the reverse situation prevails (Shugar & Wierzchowski, 1957b; Sinsheimer, 1957).

The percentage reversibility for all these compounds is quite high (90% or greater) but decreases with lengthy irradiation periods. This is most clearly exhibited by cytosine and 1-methylcytosine.

By analogy with the situation for uracil derivatives the hypothesis has been advanced that the forward reaction for cytosine derivatives also involves addition of a water molecule at the 5 : 6 double band. the

reverse reaction involving the acid-base catalyzed removal of this water molecule. A study of several uracil and cytosine derivatives in light and heavy water (Table III), in which the quantum yields for the forward

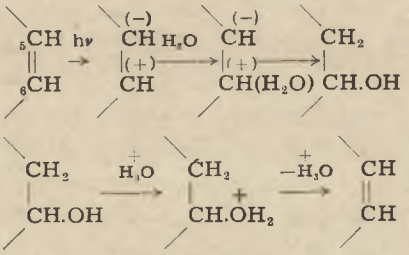
Table III

Quantum yields for photolysis of some pyrimidine derivatives in light and heavy water

Compound	$\Phi \cdot 10^3$	
	in D_2O	in H_2O
1-Methyluracil	5.7	12.5
2-Ethoxyuracil	4.0*	8.0*
Cytosine	0.7	1.7
Cytidine	4.2	9.0
2-Methoxycytosine	5.0*	10 0*
Thymine	0.9**	0.4**

* ph 7,2 phosphate buffer 0,02 M
** reaction not reversible in the dark

reaction were found to be twice as low in heavy as in ordinary water and the reverse reactions 2—3 times faster led to the following suggestion regarding the mechanism of both reactions (Shugar & Wierzchowski, 1957a):



For the reverse reaction an essentially similar mechanism has been proposed by Moore & Thomson (1957), which explains both the lower quantum yields for addition of heavy water (requiring a higher activation energy) as well as the more rapid reaction for elimination of a heavy water molecule, since in heavy water which is less basic than H_2O (Wiberg, 1955) the photoproduct will more effectively compete for the deuteron than for the proton in H_2O, as a result of which the concentration of the conjugate acid, and hence the elimination reaction, will be greater.

Despite the undoubted similarity of the reaction mechanisms for uracil and cytosine derivatives, rather striking differences are observed

between cytosine and 1-methylcytosine on the one hand and cytosine nucleosides and nucleotides on the other, both with regard to quantum yields which are approximately one order of magnitude greater for nucleosides and nucleotides (Table II) as well as changes in absorption spectrum (Figs. 4 & 5). It should be added here that the normal absorption spectra of cytosine nucleosides exhibit a maximum or a point of inflexion in the region 2300 — 2360 A, which is not evident for 1-methylcytosine (Shugar & Fox, 1952; Fox & Shugar, 1952). It therefore follows that substitution on the N_1 ring nitrogen is not the cause of the change in quantum yields or absorption spectra of nucleosides and nucleotides, or of their photoproducts, as compared to cytosine.

Studies of the dependance of quantum yields on pH (Table II) showed that for nucleosides and nucleotides Ø is practically independant of pH in the range 7 — 10.5, while below and above these pH values it decreases considerably. In acid medium Ø is practically the same for all these compounds, as is also the absorption spectrum (Fox & Shugar, 1952).

For cytosine in alkaline medium, with increasing dissociation of the carbonyl group (pK 12.2 (Shugar & Fox, 1952)) Ø gradually increases until at pH 14 it attains the same order of magnitude as for nucleosides and nucleotides in neutral medium. Under the same conditions the quantum yield of 1-methylcytosine remains unchanged. The reaction for both these compounds is, however, non-reversible, due perhaps to the instability of hydroderivatives in alkaline medium (Batt, Martin, Ploeser & Murray, 1954). Furthermore 2-methoxycytosine, which in D_2O is photolyzed with a quantum yield 50% lower than in H_2O, suggesting here too the addition of a water molecule to the pyrimidine ring (see above), is photolysed in ordinary water at neutral pH with a quantum yield similar to that for the enolized form of cytosine.

It follows from the above that the electron distribution density, in the excited state at least, in the neighbourhood of the 5 : 6 double bond in the pyrimidine ring of nucleosides and nucleotides is similar to that in the enol form of cytosine as well as in 2-methoxycytosine. The most plausible explanation is that this is the result of some intramolecular binding of the cytosine carbonyl group in cytosine nucleosides and nucleotides.

Since the dissociation constants of carbohydrates are in the range 11 — 13 and higher (see Fox & Shugar, 1952), it was proposed that the changes in quantum yield above pH 10.5 are due to dissociation of the sugar hydroxyl group (s) and the effect of this on intramolecular hydrogen bonding between these groups and the cytosine carbonyl. (Wierzchowski & Shugar, 1957). That such a mechanism is ste-

reochemically possible is shown by the studies of Clarke, Todd & Zussman (1951), Anderson, Hayes, Michelson & Todd (1954), Brown, Todd & Varadarajan (1956), Brown, Cohran, Medlin & Varadarajan (1956) and Michelson & Todd (1955) on cyclic nucleosides of uracil, thymine and cytosine in which the sugar component intramolecularly etherifies the C_2 carbonyl of the pyrimidine ring. Support for this hypothesis is provided by the fact that the quantum yields, as well as the absorption spectra, of all members of the cytosine group, are similar in acid medium.

A comparison of the quantum yields, in neutral medium, for cytidylic acids 2′, 3′, 5′, cyclic 2′ : 3′ and 5′-deoxy show that those for the two 5′ acids are the lowest as are also the extinctions of the photoproducts at 2360 Å. In addition for the two 5′ acids Ø and ε_{2360} are least for the 5′-deoxy compound. Consequently the effect of blocking the 5′ hydroxyl on Ø and on ε_{2360} of the photoproduct supports the theory as to the existence of intramolecular hydrogen bonding between the cytosine carbonyl and a carbohydrate hydroxyl, and suggests that its location is $O^2 : (OH)_{5'}$. Since, however, for both the 5′ acids the course of the photochemical reaction is qualitatively similar to that for other nucleosides, it may be assumed that here, too, we have intramolecular hydrogen bonding, not quite as strong, at $O^2 : (OH)_{3'}$ for the 5′-deoxy acid and at $O^2 : (OH)_{2'}$ for the 5′, the latter being stronger than the former (Todd et al. have thus far not found cyclic compounds of the type $O^2 : (OH)_{3'}$).

Hitherto unpublished experiments of the authors of this article on the photochemistry of isocytosine and its 3-β-d-arabinoside have shown that these too undergo reversible photolysis, with changes in absorption spectra similar to those for cytosine nucleosides and an increase in Ø in going from the base to the nucleoside which attains a factor of 25, in agreement with the fact that amino groups form stronger hydrogen bonds.

Neither 5-methylcytosine (authors, unpublished) nor 5-methyl-desoxycytidylic acid (Sinsheimer, 1957) undergo reversible photolysis, as is also the case for 2-thiocytosine. Hence substitution of the 5 or 6 positions of the pyrimidine ring, as well as the electron distribution density in the latter, are of equal importance for reversible photolysis as is the case for uracil derivatives .

Effect of concentration: The values of Ø for the reversible photolysis of pyrimidines given by various authors show reasonably good agreement. Moore & Thomson (1956), however, report a marked dependance of Ø an concentration; for uridylic acid at pH 7, Ø was found to be 0.019 at 10^{-3}M and 0.037 at 10^{-2}M. For dimethyluracil no such effect was noted.

We have been studying the influence of changes in concentration for a number of compounds and have found that in the region 10^{-3} — — 10^{-4}M a 5—10 fold change in concentration does not alter Ø for such compounds as 1-methyluracil, cytidine, cytosine, 2-methoxycytosine. The solutions were irradiated under such conditions that a portion of the incident beam was transmitted. In the case of 2-methoxycytosine we have irradiated solutions of such optical density that total absorption of incident light prevailed and under these circumstances the course of the reaction is appreciably altered, including formation of a white precipitate with the physico-chemical properties of a polymer.

Photolysis in non-aqueous media: In ethanol or methanol the photolysis of dimethyluracil is reversible, but in hexane and chloroform the changes in absorption spectrum indicate that the reaction mechanism is an entirely different one (Moore & Thomson, 1956). Because of the similarity of the reaction in water and alcohol these authors suggest that the reaction in alcohol involves addition of an alcohol molecule to the 5 : 6 double bond with the formation of an alkoxy group at the 6 position.

In addition to the use of heavy water, we have also been studying some compounds in non-aqueous media. For uracil in water-free ethanol our results are analagous to those of Moore & Thomson except that we obtain a two-fold increase in Ø, as compared to H_2O. For cytidine in anhydrous ethanol the reaction is more complicated and the changes in absorption spectrum suggest that addition to the 5 : 6 double bond, if it exists, is rather a side reaction.

Particularly interesting is the behaviour of cytosine in ethanol. The changes in absorption spectrum are completely analogous to those for 2-methoxycytosine in water at pH 7 (see Fig. 15). However in alcoholic solution 2-methoxycytosine behaves quite differently, the changes in absorption spectrum being minimal.

In connection with Moore & Thomson's sugestion regarding the addition of an alcohol molecule to the 5 : 6 double bond, in the case of dimethyluracil, it is worth drawing attention to the work of Urry, Stacey. Huyser & Juveland (1954) demonstrating the induced photochemical addition of alcohol to olefins at 2537 Å.

Complex pyrimidines: The following observation although not directly related to the context of this review, is perhaps worth noting. Rutter, Gustafson & Batt (1955) studying the photochemical behaviour of a group of derivatives synthesized by them, of the form 2-oxo-4-(aryl)-5-carbethoxy-6-trifluoromethyl-1,2,3,4-tetrahydropyrimidines, noted that only those derivatives possessing a nitro group at the 2′ position of the 4-aryl are affected, as indicated by marked chan-

ges in infra-red absorption spectra. The mechanism of this reaction was not studied in detail but, by analogy, it was assumed that the reaction involved an intramolecular rearrangement similar to that for 4-(2′-nitrophenyl)-1,4-dihydropyridines and involving reduction of the nitro group to nitroso with the simultaneous aromatisation of the pyridine ring (Berson & Brown, 1955).

Carbohydrate components of nucleosides and nucleotides

No systematic studies appear to have been made on the influence of ultraviolet irradiation on the carbohydrate moieties of nucleosides and nucleotides, aside from several observations dealing with destruction or hydrolysis of the sugar, and one short communication (Rice, 1952) dealing more specifically with this subject.

Using an unfiltered mercury lamp (characteristics not given), Rice irradiated neutral (phosphate buffer pH 7.4) solutions of ribose and glucose (2×10^{-4}M), cytidylic and adenylic acids as well as yeast RNA for periods of 60 to 90 mins. There resulted a loss of ability to reduce alkaline copper solutions. The Molisch reaction for ribose and glucose became negative; while the ability of ribose and the ribose-containing compound to form furfurol was lost.

There is no doubt from these results that both free and nucleotide--bound sugars were destroyed. However the additional observation that a protective effect obtained in the presence of substances known to protect against ionizing radiations, e.g. 1% thiourea in 1% acetic acid, indicates quite clearly that the observed destruction of the carbohydrates was the result of some indirect photochemical effect such as free radical formation and that the quartz lamp used must have emitted radiations of quite short wavelength (1849 Å).

As already mentioned above Rapport et al. (1955) reported hydrolysis of the nucleoside linkage in uridine as a result of prolonged exposure to the radiation from a germicidal lamp; while Serayda-rian et al. (1954) and Seraydarian (1955), in studies on the photolysis of DPN and TPN (see also section on purines above) demonstrated in both cases the liberation and destruction of ribose from the nicotinamide moiety, 50% of the ribose being destroyed for 100% inactivation of DPN and 30% for 90% inactivation of TPN.

However, in the case of nucleosides and nucleotides of uracil and cytosine submitted to 2537 Å radiation, sufficient to result in disappearance of the characteristic absorption maximum, the high degree of reversibility subsequently attainable shows that there is no liberation of the carbohydrate component.

It is a well-known fact that monosaccharides (aldoses) in neutral solution exhibit only end absorption in the ultraviolet below about 2300 Å, with probably a maximum in the far ultraviolet. One would therefore not expect sugars to undergo direct photolysis with radiation sources emitting wave-lengths only above 2400 Å.

In agreement with this Laurent & Wertheim (1952) observed no changes in absorption of neutral, buffered solutions of glucose, galactose, mannose and fructose when irradiated at wavelengths below 2800 Å. On the other hand Holtz (1936) claimed that solutions of glucose, fructose, arabinose and carbohydrate-like substances such as glycerol and sorbitol, buffered at pH 7.6, exhibited under the influence of irradiation characteristic absorption maxima in the region 2650—2900 A as well as the formation of high redox potential systems. Analogous changes in absorption were observed by Laurent (1956) and Laurent & Wertheim (1952) in alkaline solutions of various monosaccharides subjected to irradiation from a GE UA-3 mercury arc (2540—3650 Å). The reaction products, at pH 12, exhibited absorption maxima from 2670 Å (glucose, galactose) to 2520 Å (d-xylose) the extinction of which was dependant on the sugar concentration, pH, temperature (higher with increase in temperature), and the atmosphere in which irradiation was carried out (in N_2 more photolysis products than in O_2). The photolysis product of glucose exhibited a maximum at 2450 Å in acid solution and a pK of 4.5.

Since, however, analogous changes may be observed in solutions of monosaccharides and polysaccharides under the influence of alkali alone, albeit at a lower rate, and exhibiting the same dependance on pH and temperature (see Petuely & Meixner, 1953 and literature cited therein); as well as in aqueous solutions under the influence of γ-irradiation (Henoh, 1955), the above observed effects are certainly not due to any primary photochemical reactions.

Shugar & Wierzchowski (1957a) photolysed alkaline solutions of adenosine (10^{-4} M in 0.01 M NaOH) with radiation from a resonance lamp filtered to eliminate completely wavelengths below 2400 Å. After 1 hour's irradiation at an intensity of 10^{17} quanta/cm^2/min. there was no change in the content of ribose as determined by the orcinol reaction.

It therefore seems reasonable to conclude that reports of the destruction of carbohydrate components of nucleosides and nucleosides are indicative of the participation of radicals. In some instances it is conceivable that such destruction may occur through energy transfer from the aromatic ring via the glycosidc linkage, but further studies are necessary to establish such a mechanism.

Nucleic acids

Qualitatively the photochemical behaviour of nucleic acids roughly parallels that to be expected from the behaviour of the individual components if due account is paid to the modifications resulting from internucleotide linkages.

Irradiation of DNA results in a decrease in viscosity and streaming birefringence which was interpreted by Hollaender, Greenstein & Jenrette (1941) as evidence of depolymerization. While this may be so for prolonged irradiation, loss of viscosity alone is hardly evidence of depolymerization since this can result from heating or acidification without change of molecular weight. Giese (1947) found that the long nucleoprotein fibres of sea urchin sperm, formed upon transferance from 2 M salt solution to distilled water, decrease in length under the influence of irradiation and following prolonged exposure no longer form fibres but only a murky solution. Since, however, the nucleoprotein was irradiated in saline solution, where it is strongly dissociated, the primary effect dealt with here is on DNA and not nucleoprotein.

Seraydarian, Canzanelli & Rapport (1953) irradiated RNA and DNA at concentrations of 0.1 mg./ml. at pH 6.8 in shallow pans (depth of solution 1 cm.) with a bank of 7 G. E. germicidal lamps placed a few mm. above the surface of the solution. Following irradiation the solutions were concentrated and titration curves run on them. For short periods of irradiation (about 2 hours) very little change was discernible other than those involving the purine and pyrimidine rings. With prolonged irradiation there resulted a rupture of internucleotide linkages and finally a release of about one-third of the total phosphate as inorganic phosphate. The pyrimidine rings were found to be the most labile, in agreement with other observers. The absorption spectrum was found to diminish about one-sixth to one-third before internucleotide linkages were affected.

In Fig. 6 are exhibited the variations in viscosity and extinction resulting from the irradiation of DNA at neutral pH (Errera, 1952a 1952c). Two types of sources were used in this work, a British Thermal Syndicate resonance lamp and a high-pressure, Philora lamp, but the results obtained were essentially similar. An attempt was made to calculate the quantum yield, based on the decrease in viscosity and using a calibration curve of viscosity vs. DNA concentration; the value obtained in this way is very low, about 2×10^{-6}, assuming a molecular weight of 10^6 for the DNA molecule. Using the results of Hollaender et al. (1941), the author obtained a value for Ø closer to 10^{-5}, which could be due to some difference in the DNA preparation. For desoxyri-

bonucleoprotein the results were quantitatively similar, but since irradiation in this case was in saline solution, where the nucleoprotein is dissociated, the primary effect probably involved only the DNA component, as in the experiments of Giese (1947) cited above. On the basis of some qualitive observations in dilute salt solution, where the degree, of dissociation is lower, it was concluded that the behaviour of the DNA is not markedly dependant on whether it is free or combined with protein.

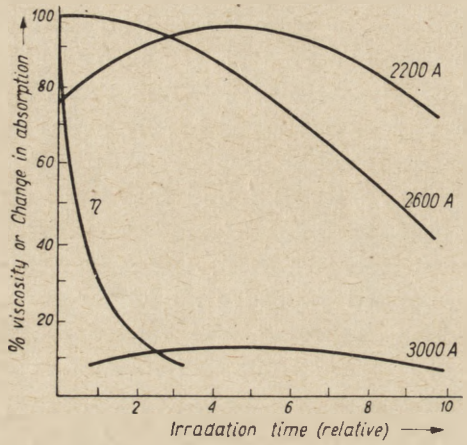


Fig. 6. Variations in extinction at different wavelengths (2200, 2600, 3000 A) and in viscosity (η) of DNA at various concentrations up to 0.6 mg/ml during the course of ultraviolet irradiation (Errera, 1952 a)

With prolonged irradiation to the point where only 70% of the pyrimidines could be recovered from a hydrolyzate of irradiated DNA, the purines were recovered almost intact. It is unfortunate that no attempt was made to determine the proportions of thymine and cytosine in the recovered pyrimidines, in view of the large differences in quantum yields between these bases as well as between their nucleotides (see Tables I & III).

Extensive irradiation of DNA also leads to the appearance of a small percentage of dialyzable products while about 10—15% of the photolysed DNA is no longer precipitated by acetone (Errera, 1952b). However the electrophoretic mobility of the remaining 85—90% of the DNA is unchanged. Under similar conditions of irradiation the solubility of desoxyribonucleoprotein in 0.14 M NaCl is slightly increased.

Using as a criterion the decrease in extinction of the absorption maximum at 2600 Å, Christensen & Giese (1954) found the sensitivity of DNA to irradiation to be greater at acid than at neutral pH. This is to be contrasted with the observation that quantum yields for cytosine nucleosides and nucleotides are appreciably lower at acid than at neutral pH (Wierzchowski & Shugar, 1957), and suggests that the decrease observed was not necessarily due only to photolysis of the bases.

Irradiation of rat thymocytes at very high energies with an unfiltered Hanovia resonance lamp results in a 50% decrease in the amount of polymerized DNA which can subsequently be extracted, as compared

to non-irradiated thymocytes. The irradiated and control DNA samples exhibited no differences with respect to relative or structural viscosities and sedimentation rates; but the large amounts of thymine present in the supernatant fluids of the irradiated sample (collected during the extraction procedure) testified to the extensive fragmentation resulting from irradiation (deCourcy, Ely & Ross, 1953) and suggests, by comparison with the results of Errera (1952b, 1952c) that DNA *in vivo* is at least as susceptible, if not more so, to irradiation as *in vitro*. On the other hand greater yields of RNA have been obtained from irradiated than from non-irradiated yeast cells (Loofbourow, 1948).

Following irradiation of a 0.3% solution of DNA at neutral pH to the point where the viscosity is reduced to 26% of its initial value, Devreux, Johannson & Errera (1951) applied the staining technique of Kurnick (1950a) to show that the staining affinity of the acid was decreased by about 30%. This does not necessarily indicate degradation of the molecule since even heat „denaturation" of DNA, which is accompanied by no change in molecular weight, results in a decreased affinity for methyl green. In acid medium (pH 4.1) the decrease in staining ability following photolysis is even more pronounced (cf. Christensen & Giese, 1954, above).

Making use of the quantitative cytochemical method of Kurnick (1952b) for methyl green staining of DNA, Errera (1952c) also demonstrated that the affinity *in situ* of cell nuclei for methyl green decreases as a result of irradiation, the decrease being approximately logarithmic with respect to dose. The Feulgen reaction is unaltered. Attempts to correlate the amount of energy required *in situ* with that *in vitro* for reduction of staining affinity gave rather discordant results: about 50 times less energy was required *in situ!* Startling as is this difference, it cannot be discounted without further data along these lines. In most experimental work little or no consideration has been given to the difference in state of DNA in solution and *in vivo*. Of interest in this connection are some experiments of Setlow & Doyle (1953, 1954) on the irradiation of dry films of DNA, which were found to form a gel in aqueous solution under the influence of irradiation. The efficiency of gel formation was found to parallel the DNA absorption spectrum over the range 1850—3000 Å and the quantum yield for the process throughout this spectral range was about 10^{-2}. This value is 2 to 3 orders of magnitude greater than for other properties of nucleic acids in solution.

Kaplan (1955b) has studied this phenomenon in greater detail and demonstrated that the rate of ultraviolet gelation of DNA films is markedly dependant on the relative humidity, the rate increasing by a fac-

tor of about 22 as the humidity is decreased from 97.5% to 33%. Extending the results of a previous investigation (Kaplan, 1955a), the same author showed that a quantitatively similar relationship prevailed for the s-mutation and killing rates of *Serratia marcesens* as a function of relative humidity, from which it was concluded that a common mechanism exists for all three processes involving hydrogen-bond rupture in the DNA molecule. These findings are in agreement with the high quantum yields reported by Setlow & Doyle (1953, 1954) for irradiation of dry DNA films and are also of considerable interest in connection with the state of the DNA molecule in living cells.

Oster & McLaren (1950) have studied the degradation of RNA from TMV, obtained from the virus by heating the latter; such RNA has a molecular weight of about 15.000 and an intrinsic viscosity of about 13, corresponding to an axial ratio of about 10 for a rod. Prolonged irradiation of a 0.3% solution resulted in a drop of intrinsic viscosity to practically zero, suggesting breakdown to smaller molecules. Simultaneously the optical density of the absorption maximum increased 10%. A similar increase in optical density was noted on irradiation of a dilute solution of the same RNA. Furthermore the RNA failed to stain metachromatically with safranin in solution after irradiation. This is the only instance known to us where irradiation of nucleic acid is accompanied by an increase in the absorption maximum, and the result is at variance with the later findings of one of the authors (McLaren & Takahashi, 1957) on infectious RNA from TMV (see below).

Of much greater interest are those experiments where changes in physical and physico-chemical properties may be correlated with changes in biological properties. If we exclude for the moment viruses (see below) only two such examples are known to us, but these are quite instructive and it is to be hoped that more will become available shortly.

The inactivation of infectious RNA from TMV occurs without any reduction in intrinsic viscosity, but with a small decrease in optical density of the absorption maximum at 2600 A of about 0.7% which, according to the calculations of the authors (McLaren & Takahashi, 1957) corresponds roughly with that to be expected from the known quantum yields for uridylic and cytidylic acids.

For DNA exhibiting transforming activity, the dose required to produce an observable decrease in viscosity, 2×10^5 ergs/mm^2, is about 500 times as great as that required to decrease the activity to 10% of the original (Zamenhof, Leidy, Hahn & Alexander, 1956).

From these two examples alone it follows that the doses necessary to provoke important biological effects are considerably lower than

those resulting in degradation of polynucleotide chains and that the biological effects are probably localized in character. From the known quantum yields for purines and pyrimidines it is likely that such localization is confined to the pyrimidines.

Reversibility with nucleic acids

In view of the relatively high resistance to irradiation of the purines as compared to pyrimidines, together with the fact that for some of the latter the reaction is reversible, it is quite conceivable that irradiation of nucleic acids which does not result in extensive damage to the nucleotide chains may affect primarily those pyrimidine components the photolysis of which is reversible. Since reversible photolysis of pyrimidine nucleotides involves substitution only of the 5 and 6 positions of the pyrimidine ring, which are not involved in any of the internucleotide linkages in polynucleotide chains, one can even envisage the possibility of „inactivation" of nucleic acids as being due almost solely to this effect. The almost 100% recovery of purines from irradiated DNA, *in vitro*, (E r r e r a, 1952a), is in agreement with such a conception.

In seeking a parallel with the phenomenon of photoreactivation, it is necessary to visualize how such „inactivation" may be reversed in living cells. The fact that photoreactivation is usually incomplete suggests that, if it is due to reversible photolysis of pyrimidine nucleotides, the primary effects of irradiation could not have been limited to the pyrimidine rings alone but may have, for example, resulted in rupture of secondary internucleotide linkages by energy transfer along the chains. However, such effects must be very subtle indeed, since after 100% inactivation of TMV or infectious RNA from TMV the resulting physico-chemical changes in these molecules are very slight.

Photoreactivation is usually achieved by exposure to visible light and this undoubtedly occurs by energy transfer from non-nucleotide components. W e l l s (1956) has actually demonstrated that the ultraviolet inactivation of DPN *in vitro* may be reversed by photosensitization. The activation energy required for reversal of photolysis in the case of pyrimidines is not very high, about 22 kcal./mole for 1,3-dimethyluracil (M o o r e & T h o m s o n, 1956) and for some of the nucleotides proceeds spontaneously at room temperature. The fact that more elevated temperatures helps along this reaction is in accord with the observed reversal of photolytic effects *in vivo* in a number of instances merely by increasing the temperature after irradiation (A n d e r s e n, 1951).

Theoretically, therefore, one may envisage biological photoreactivation as a manifestation of the reversible photolysis of the pyrimidine

nucleotide components of nucleic acids. Some preliminary observations in support of such a hypothesis (Wierzchowski & Shugar, 1956; Shugar & Wierzchowski, 1957a) have been extended (Shugar & Wierzchowski, 1957b).

The photochemical behaviour of RNA and APA demonstrate that the reversible photochemical changes undergone by cytosine and uracil nucleotides likewise occur to these nucleotides when they are incorporated in polynucleotide chains. If a solution of RNA in 0.02M phosphate buffer pH 7 is irradiated to the point where the drop in extinction at 2600 Å is still 50% that of the initial extinction, then a few minutes heating in the temperature range 50—80°C suffices to restore 50% of the drop in absorption (Fig. 7). More prolonged irradiation leads to a decrease in the percentage recovery upon heating. As can be seen from the absorption spectra following irradiation, there is at first little change below 2400 Å, following which the height of this portion of the spectrum increases slightly, but does not diminish upon heating.

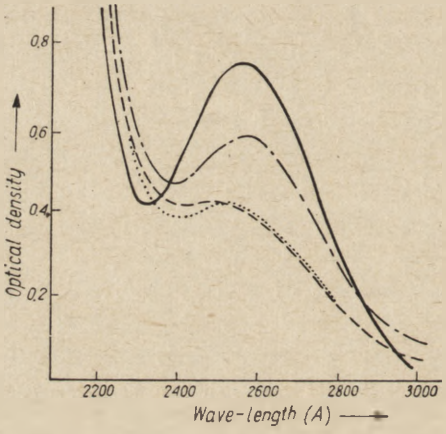


Fig. 7. Irradiation and subsequent partial reversibility for commercial sample of RNA (Merck) in 0.002 M phosphate buffer pH 7, Thermal Syndicate resonance lamp without acetic acid filter.
Curves: ——— before irradiation, — — — — after 165 min photolysis, ... after 18 h at room temp., —.—.—.— after 5 min at 85°C

In order to obtain results which might be more readily interpreted, some experiments were carried out with APA and desaminated APA and RNA, the acids being desaminated by the method of Bredereck as outlined by Vandendriesche (1951). In the case of APA the polynucleotide chain contains only thymidylic and desoxycytidylic acids; since the quantum yield of the former is one order of magnitude lower than for the latter, it may be assumed that for low irradiation doses photochemical effects will involve only the cytosine ring.

The behaviour of two different preparations of APA (Durand & Thomas, 1953; Tamm, Hodes & Chargaff, 1952) in neutral solution are similar both as regards reaction rate and change in absorption spectra (Fig. 8). In the short wave-length region of the spectrum the changes which occur are similar to those for RNA.

If we assume that the absorption spectrum of desoxycytidylic acid is not markedly altered as a result of incorporation in the polynucleo-

tide chain and that it is only this component which is affected by low irradiation doses, we can calculate approximately the quantum yield for APA. The value we obtain in this way is the same as that for cytidylic acid 2′ or 3′, or about threefold that for desoxycytidylic acid. In our opinion this indicates that the postulated hydrogen bonding between the carbonyl cytosine and one of the sugar hydroxyls is stronger for desoxycytidylic acid when it is incorporated in the polynucleotide chain than when it is in the free state.

Chromatographic studies of APA, following irradiation to the point where a 50% decrease in absorption has taken place, showed that the polynucleotide chain was still intact since no small fragments could be detected.

In acid and alkaline media photolysis produces the same type of changes in absorption spectra as in neutral medium, but the reaction rate is 30—40% lower. At alkaline pH the reaction may still be reversed by heating; but there is no reversibility in acid medium.

Identical behaviour was exhibited by both APA preparations used despite the fact that one of them, that prepared according to Durand & Thomas (1953) differs from the preparation of Tamm et al. (1952) in certain properties, in the latter acid it has been shown that the cytosine amino group is free, while in the former the behaviour of this group is somewhat anomalous. (Shugar & Adamiec, 1957).

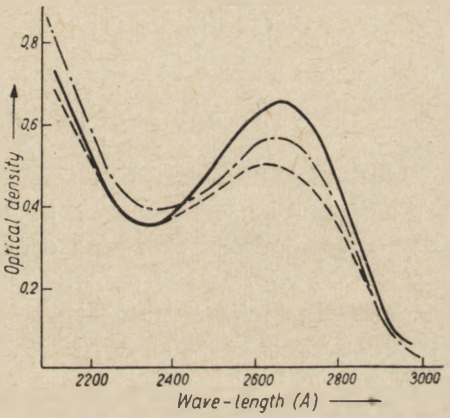


Fig. 8. Irradiation of apurinic acid in 0.02 M phosphate buffer pH 7.0 followed by heating of the photoproduct. Curves: ——— before irradiation, — — — — after 30 min photolysis, —.—.—.— after 10 min at 92°C

On the other hand for desaminated APA (unpublished) and RNA where the first contains thymidylic and desoxyuridylic acids and the second only uridylic acid as well as xanthine and hypoxanthine, experiments conduced under identical conditions exhibited no evidence of reversibility, a result somewhat surprising.

The changes in absorption spectrum of irradiated desaminated APA are similar to those for free uridylic acid, and the quantum yield approximately that to be expected. For desaminated RNA the change in absorption spectrum initially corresponds to that expected for the rapid photolysis of uridylic acid, then slows down; while the absorption spe-

ctrum remaining, and quite resistant to irradiation, exhibits two maxima corresponding to a mixture of xanthine and hypoxanthine.

The failure to observe reversibility with both desaminated acids is difficult to reconcile with the almost 100% reversibility exhibited by free uridylic acid and the good degree of reversibility obtained with RNA and APA. It is, of course, not inconceivable that the desamination reaction itself may have resulted in some modification of the polynucleotide chains, although in the case of RNA one would not expect this from the results of Vandendriessche (1951). Of some singnificance in this connection may be the results for 4-ethoxyuracil and 2,4-diethoxypyrimidine, for neither of which does reversibility prevail and for both of which the quantum yield is relatively low (Table I).

Further studies along these lines could obviously much more profitably be made on model di- and tri-nucleotides, as well as on enzymatically prepared polynucleotides of controlled composition (Grunberg-Manago, Ortiz & Ochoa, 1955).

Receptors of radiation in living organisms

Most of the evidence pointing to nucleic acids as the immediate receptors of radiation resulting in a variety of biolgical effects is derived from investigations of action spectra. It must be emphasized, however, that action spectra are not always unambiguous and there is a frequent tendency to tacitly assume that an action spectrum with a peak in the neighbourhood of 2600 A indicates that nucleic acids are the only receptors.

The precautions necessary for adaquate interpretation of action spectra have been discussed in detail by Loofbourow (1948) and restated by Blum (1950). Particularly doubtful are those cases where the measurements are based only on comparisons of effectiveness of incident doses at the different wavelengths used. In the case of bacteria and viruses, for example, the very high absorption of the nucleic acid component by comparison with that of the protein moiety is such that the action spectrum will of necessity resemble that of a nucleic acid spectrum; but if radiation absorbed by the protein components has an effect of the same order of magnitude, the action spectrum may not distinguish between the two. Greater confidence can be attached to the results when the action spectrum is based on actual quantum yields but this, unfortunately, is possible for only some of the simpler systems such as viruses, provided that adequate corrections for scattering are made to the absorption spectrum.

A further requirement is that the quantum yield be independent of wave-length. For nucleic acids and their derivatives there is very little data regarding dependance of Ø on wave-length; Setlow & Doyle (1954) found that DNA irradiated in the dry state forms a gel in aqueous solution and that the Ø for this process is constant over the range 1850—3000 Å. For proteins considerably more data is available, showing not only that variations of Ø with wave-length exist (McLaren, 1949) but that in some instances at least such variations may be appreciable (Setlow & Doyle, 1957). On the other hand for two bacteriophage strains (T_1 and T_2), measurements conducted independently in two laboratories showed that quantum yields are reasonably independant of wavelength over the range 2200 — 3000 Å. (Zelle & Hollaender, 1954), as can be seen from Fig. 9.

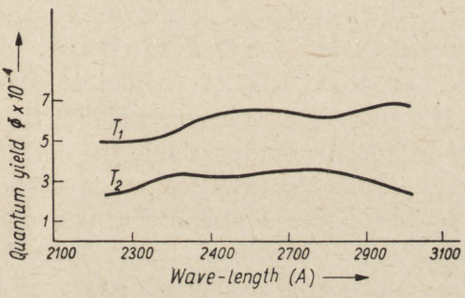


Fig. 9. Quantum yields for bacteriophage T_1 and T_2 at different wavelengths. Each curve is the mean of observations made in 2 different laboratories (Zelle & Hollaender, 1954)

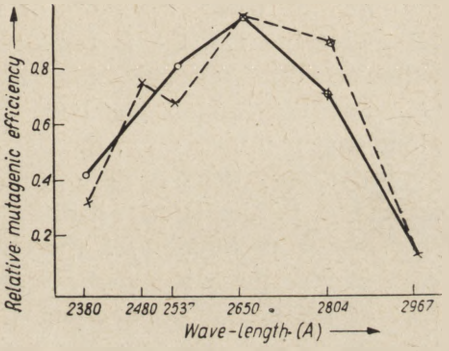


Fig. 10. Ultraviolet action spectra for mutation production in *E. coli SD-4* (— — —) and Pur (———) according to Hollaender & Zelle (1954)

Fig. 10 shows action spectra for mutation production in 2 strains of *E. coli* and presented by the authors (Hollaender & Zelle, 1954) as exhibiting typical nucleic acid maxima. It seems to us that too few points have been plotted here to distinguish between nucleic acids and nucleoproteins with any degree of certainty, and that on the basis of these curves alone participation of proteins cannot be excluded. A similar situation prevails with regard to many other published action spectra due, in part, to the experimental difficulties of obtaining a sufficient number of lines with suitable intensities in the region 2600—2900 Å. Fig 11 (p. 281) is an example of an action spectrum (for irradiation of bacteriophage M-5 in the dry state) where special attention has been paid to the region in the neighbourhood of 2800 Å and from which it appears reasonably evident that the protein component is involved, although perhaps to a lesser extent than the nucleic acid portion.

Wyss (1954) has suggested that a considerable part of the biological action of ultraviolet on bacteria may be due to the production of peroxides and associated oxidizing species in the surface lipids and that the similarity between action spectra and nucleic acid absorption is purely coincidental. It is true that the production of peroxides could result in very drastic effects on nucleic acids (see below) but this would apply to other important cell components as well. It cannot be said that such a view has gained wide favour.

In general the preponderance of evidence does point to the important role of nucleic acids as the main receptors of biologically effective radiation in the ultraviolet, with this qualification, that less is known about the precise extent to which proteins are involved.

Photoreactivation has been proposed as an additional tool for pinpointing the direct receptors of radiation. That the site at which photoreactiveable damage is produced is a nucleoprotein is shown by the action spectra for bacteria, plant viruses and yeast (Swenson & Giese, 1950) and it has been further proposed that it is nucleic acid alone that is involved, on the basis of Hershey & Chase's (1952) finding that most of the protein portion of bacteriophage is outside the cell it infects. Fluke (1951) has, however, demonstrated that the action spectra for production of reactiveable and non-reactiveable phage are very similar, with a major peak at 2650 Å and a minor one at 2800 Å. Blum (1954) has pointed out that a number of effects of ultraviolet light on living cells are not reversible and suggested that photorecovery may be used as one of the criteria for determining whether the initial effect of irradiation is on the nucleic acid components or not.

The suggestion of Blum undoubtedly derives support from the experimental observations that photolysis *in vitro* of pyrimidine nucleotides, and to some extent of polynucleotide chains, is reversible. Further supporting evidence is provided by a comparison of photoreversibility for nuclear and cytoplasmic effects. Several observers have presented evidence in support of the conception that the site of photoreversible ultraviolet-induced injury is the cell nucleus (see Brandt & Giese, 1956). The latter authors studied the effect of various wave-lengths in the ultraviolet on immobilization and on division delay in the protozoan *Paramecium caudatum*. For the former the action spectrum is protein-like in character, while for division delay it is nucleoprotein in shape. In addition it is believed that the immobilization effect is localized in the cytoplasm and division delay in the nucleus. The two effects were separated, as regards primary injury, by using for immobilization studies wave-lengths below 2400 Å which are sufficiently well absorbed by the cytoplasm so that they do not reach the nucleus. It was found in this

way that division delay, with its nucleoprotein-like action spectrum, is reversible; while immobilization is not.

A recent observation by Zill (1957) points to the participation of a free nucleotide as a primary receptor. In view of the demonstrated involvement of uridine diphosphate glucose in sucrose synthesis by plants, the photolysis of this compound was studied and it was found that rate of loss of absorption maximum with time is of the same order of magnitude as that required to inhibit sucrose synthesis in plants upon exposure to ultraviolet light of the same intensity. This is regarded by the author as supporting evidence for inhibition of sucrose synthesis as a result of UDPG destruction. In view of the same author's demonstration of reversibility of UDPG photolysis, it would certainly be of interest to examine the possibility of reversal of inhibition of sucrose synthesis. Photoreversibility of ultraviolet radiation injury to higher plants has actually been demonstrated in two cases (Bawden & Kleczkowski, 1952; Tanada & Hendricks, 1953).

Kinetics of inactivation

The photochemical inactivation or destruction of molecules, as well as a variety of biological systems, exhibiting biological activity frequently follows a first-order course with respect to time or irradiation dose so that

$$\ln \frac{C}{C_0} = -kt$$

where C_0 is the initial concentration or activity, C the activity at time t and k the first-order rate constant, or

$$\ln \frac{C}{C_0} = -aI$$

where I is the total energy incident on the sample irradiated and a a constant dependant on the energy absorbed and the quantum yield for the process. This has generally been taken to indicate that the process involved is of the "one-hit" type, i.e. that although many quanta may be absorbed without inactivation, that when inactivation does occur it is due to the absorption of a single quantum or, in other words, to one "hit".

There are, however, instances where the course of inactivation is not strictly first-order, the survival curve being sigmoidal in shape, and the process is then interpreted as of the "multiple-hit" type, so that inactivation requires several hits. The general formula for such a process which requires m inactivating hits for each of N necessary parts of the molecule, with a probability a per unit total dose applied is (Timofeeff-Ressovsky & Zimmer, 1947).

$$\frac{C}{C_0} = 1 - \left| 1 - e^{-aI} \sum_{0}^{m-1} \frac{aI}{m-1} \right|^N$$

If only one necessary part of the molecule requires one inactivating "hit", the formula reduces to

$$\frac{C}{C_0} = e^{-aI}$$

which is the same as the lst order equation given above. A typical inactivation curve for a "multiple-hit" type of process is that for agglutination activity of influenza virus (Fig. 13, p. 284).

While in the case of ionizing radiations considerable supplementary evidence has been adduced in support of the one-hit type of process for those cases where the survival curves are logarithmic, the same cannot be said to hold for irradiation with ultraviolet light (Lea, 1955). One rather potent argument against this is the fact that enzymes and viruses, following irradiation with ultraviolet light, and even in those cases where the course of inactivation is strictly logarithmic, are rendered more labile to other inactivating agents. It is a well-known fact that partially photolysed enzymes and proteins are much more sensitive to heat than non-irradiated controls. A similar situation applies to TMV (Oster & McLaren, 1950) and other viruses and bacteriophage (Kleczkowski, 1954; Kleczkowski & Kleczkowski, 1953), a fact which has been taken advantage of in the preparation of vaccines (see below). It applies also to the physico-chemical properties of DNA (Butler & Conway, 1953; Butler, 1954) as well as to DNA transforming activity (Zamenhof et al., 1956) for the latter of which the orginal inactivation curve is of the "multiple-hit" type. Kleczkowski (1954) has shown that this "weakening" of the activity of an enzyme or virus, without complete inactivation, is not due to the effect of inactivated on active molecules.

Furthermore it should be pointed out that, for the irradiation-induced formation of mutants, an equally good fit to a logarithmic dependance of rate on dose may be obtained theoretically if each inactivation is assumed to be the result of a number of random events (Opatowski, 1950; Opatowski & Christiansen, 1950).

Coming now to the question of quantum yields, it is clear that in those cases where we are dealing with sigmoidal survival curves it is difficult to ascribe any definite meaning to Ø; although it should be noted that Atwood & Norman (1949) have suggested an interpretation of such survival curves on the basis of the exponential inactivation of the individual units of a multiunit system, their procedure permitting of the calculation of Ø for the individual units.

However, even where the survival curve is exponential, the above considerations show that there is at least some doubt, in those cases where we are dealing with macromolecular systems, as to whether the term quantum yield has a unique meaning and refers only to changes (physical or biological) brought about by a single quantum.

These arguments, nonetheless, in no way detract from the usefulness of quantitative measurements of Ø where logarithmic survival curves prevail. That such values of Ø do have some definite meaning is well illustrated by the few examples given in this review of an approximate correlation between quantum yields for nucleic acids on the one hand and pyrimidine nucleotide components on the other (Shugar & Wierzchowski, 1957b; McLaren & Takahashi, 1957).

Energy transfer in irradiation effects

Assuming that the immediate receptors of radiation are nucleoproteins we are still faced with the problem of determining whether the effects of irradiation occur directly at the nucleic acid or protein components (or both) or whether these serve also as channels for funneling the absorbed energy to some other cell components in living cells. Not much direct information exists on this score although an increasing interest is being shown towards this problem.

In the case of proteins several examples exist of the transfer of light energy between groups separated by distances greater than the effective collision diameters. Bucher & Kaspers (1947) observed the dissociation of carbon-monoxymyoglobin by light of wavelength 2800 Å (absorbed by the aromatic amino acids) with the efficiency of light absorbed by heme itself. Bannister (1954), measuring the efficiency of light absorbed by the protein component of phycocyanin in exciting fluorescence of the chromophoric group, concluded that all quanta absorbed, in the range 2537—4043 Å, are equally effective. Broser & Lautsch (1956) coupled poly-dl-(phenylalanine-glutamic acid) with carbonyl-mesohemin-IX and irradiated this complex at a frequency corresponding to the absorption peak of phenylalanine; this resulted in dissociation of CO from the hemin group with a $Ø \approx 1$, and was interpreted as indicating highly efficient energy transfer along a polypeptide chain due to overlapping amplitude functions of neighbouring groups in a helix. But an equally conceivable interpretation in the absence of further data is resonance transfer (Shore & Pardee, 1956b).

An illustration of energy transfer from a nucleotide-containing coenzyme (DPN) to a protein to which it is bound has been demonstrated in the case of triosephosphate dehydrogenase (Shugar, 1951a, 1951b),

the result being an increase in enzymatic activity due to reduction of inactive —S—S— groups to —SH. An analagous phenomenon *in vivo*, resulting in the appearance of newly-formed —SH groups (Calcutt, 1950), has been interpreted on the basis of the above mechanism (Shugar, 1951a). Attention has also been drawn to the possible role of this observation in the phenomenon of photoreativation (Dulbecco, 1955).

An extremely interesting study on electronic energy transfer by proteins and nucleic acids is that of Shore & Pardee (1956b) who prepared several protein-dye and nucleic acid-dye conjugates and measured the effectiveness of light absorbed by the protein or nucleic acid in exciting fluoresence in the attached dye. Considerable transfer of energy by proteins was observed and this was found to be roughly proportional to the fluorescence efficiency of the protein itself, such fluorescence indicating the existence of excited lifetimes sufficiently long to permit of resonance transfer of energy. However, in the case of both RNA and DNA, in neutral solution, no such transfer to the attached dye could be observed, and this was attributed to the non-fluorescent nature of nucleic acid derivatives in solution (Shore & Pardee, 1956a). There is, however, some conflicting evidence on this latter point and it is perhaps worth while summarizing this in view of its importance in resonance transfer of energy (Karreman & Steele, 1957).

Heyroth & Loofbourow (1931) and Stimson & Reuter (1941) reported that a number of purine and pyrimidine derivatives fluoresce, but an examination of the experimental techniques used, particularly by the latter authors, raises some doubts as to the validity of the measurements. Jacobson & Simpson (1946) examined a number of purine derivatives and reported that only guanine and uric acid showed weak fluorescence. Duggan, Bowman, Brodie & Udenfriend (1957), who examined a wide variety of purine and pyrimidine derivatives, found no pyrimidines to exhibit fluorescence but did obtain positive results for several purines. On the whole it does seem established that some of the natural purines, including guanine, do exhibit fluorescence.

From a study of the fluorescence efficiency of TMV as well as its protein moiety alone Shore & Pardee (1956b) concluded that energy transfer from protein to nucleic acid is probably nonexistent but that up to 25% transfer of energy from nucleic acid to protein may exist. Indirect, but of course not conclusive, evidence of low efficiency of energy transfer from nucleic acid to protein is the well-known fact that virus infectivity may be frequently destroyed by irradiation without appreciably affecting the antigenic properities of the virus.

Clearly further experiments along these lines are to be desired. Recent reports dealing with the various modes of energy transfer include those of Bucher (1953) and Magee, Kamen & Platzman (1953). Karreman & Steele (1957) have summarized the literature on, and discussed in detail the theoretical basis of, energy transfer in biological systems by resonance, i.e. the transfer of energy by electrodynamical interaction from an excited oscillator to an oscillator in resonance with it and so close to it that their separation is small by comparison with the wavelength of the vibrating electromagnetic field emitted by the former.

Viruses

Photochemical techniques have been widely applied to studies on the properties of viruses and have also found practical application in this field for the preparation of vaccines and serum products. Some of the earlier observations on ultraviolet inactivation of viruses are included in a review by McLaren (1949).

Viruses are eminently suitable systems for photochemical studies because of their relatively simple composition by comparison with other systems exhibiting biological activity..... in effect they are nucleoproteins exhibiting biological activity which is dependant on the integral structure of the molecule. Furthermore they offer the additional advantage that they may be irradiated *in vitro* as well as in the intracellular state where they behave as components of more complex integrated biological systems.

An important factor in photochemical studies on viruses is the manner of testing for activity, in view of the phenomenon of photoreactivation. As a result of this Luria (1955) defines an inactive virus particle as one that is unable to parasitize a host cell or which, upon parasitizing a host cell under conditions where no reactivation occurs, fails to give rise to the production of active virus.

In most instances the ultraviolet inactivation of viruses is a first-order reaction or closely approximating to it.* For a number of viruses this has been stablished with good accuracy, e. g. for TMV (Oster & McLaren, 1950), other plant viruses (Bawden & Kleczkowski, 1953), bacteriophage M-5 (Franklin et al., 1953). Some exceptions have been reported for several bacteriophage (Benzer et

* At very low survival ratios several observers have reported a tendency for the inactivation curve to level off. For coliphage T_1 & T_2 this has been ascribed by Hollaender & Zelle (1954) to multiplicity reactivation (Luria, 1947).

al., 1950), although the reasons for deviation from a first-order law are not clear. For a number of actinophages Welsch & Minon (1955) find the reaction to be logarithmic only after an initial phase with a higher inactivation rate. A similar behaviour was noted by Fogh (1955) for one strain of poliomyelities virus, which in this case was interpreted as due to the presence of 2 strains of different sensitivity.

In a few instances quantum yields for inactivation have been measured and such values are tabulated in Table IV. Zelle & Hollaender (1954) point out, however, that for phages T_1 and T_2 the absolute values of Ø cannot be considered as definitely established because the number of active phage particles per plaque-forming unit are not accurately known, while corrections for scattering were not sufficiently good to establish the ultraviolet spectrum with sufficient accuracy. The same authors draw attention also to the fact that Luria (1953) obtained sigmoidal survival curves for T_2 at 2537 Å which, if true, would invalidate their quantum yields. It should also be noted that for T_1 it has been claimed by Eckart (1954) that the reciprocity relationship does not hold at low irradiation intensities (at 2650 Å), although this is in contradiction to the results of Fluke & Pollard (1949). Some of these discrepancies might be due to differences in experimental conditions (see Zelle & Hollaender, 1954, for discussion).

Table IV

Quantum yields for inactivation of phage and viruses

Virus	Irradiation wavelength (A)	Ø	Reference**
TMV	2537	2.6×10^{-5}	(1)
	2537	7×10^{-5}	(2)
	2537	4.3×10^{-5}	(3)
	2537	$3 - 6 \times 10^{-5}$	(4)
T_1 phage	2220 — 3022*	6×10^{-4}	(5)
	2600	3×10^{-4}	(6)
T_2 phage	2220 — 3022*	3×10^{-4}	(5)

* Sie also Fig. 9.

** References (1) Über (1941)
(2) Lea (1955)
(3) Oster & McLaren (1950)
(4) Kleczkowski (1954)
(5) Zelle & Hollaender (1954)
(6) Fluke (1953)

Action spectra for various viruses have shown that nucleic acids are the principal receptors of radiation leading to inactivation (Loofbourow, 1948; Hollaender & Oliphant, 1944, Hollaender & Zelle, 1954). But more detailed examinations of action spectra indicate that the protein component plays some role as well (Franklin et al., 1953, Fluke, 1953) as is evident from Fig. 11.

The sensitivity of viruses to irradiation is not directly related to nucleic acid content. Potato virus X, the nucleic acid content of which is the same as that of several strains of TMV, is more sensitive than the latter, while different strains of TMV differ appreciably in sensitivity (Siegel & Wildman, 1954. Bawden & Kleczkowski, 1955). The conclusion of the latter authors is that the nucleic acids of different viruses differ in sensitivity to inactivation by ultraviolet light and it is interesting to note in this connection that Reddi (1957) has demonstrated differences in structure of the ribonucleic acids from different strains of TMV. On the other hand Siegel, Wildman & Ginoza (1956) examined the susceptibility to loss of infectivity of infectious RNA isolated from two different TMV strains according of the method of Fraenkel-Conrat & Williams (1956) and found no difference in photochemical behaviour (Fig. 12).

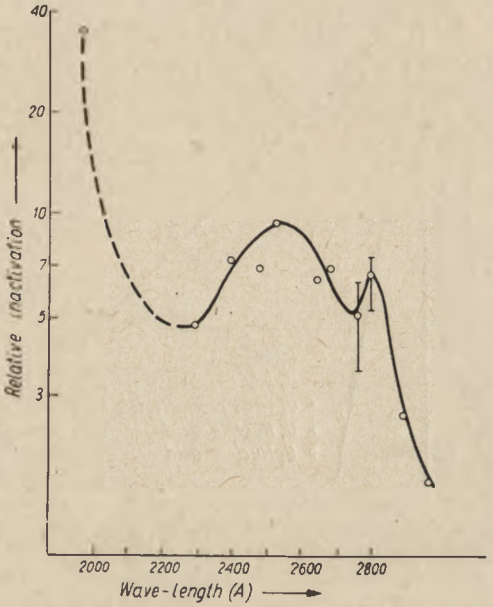


Fig. 11. Action spectrum for *Bac. meg.* bacteriophage *M-5* (Franklin, Friedman & Setlow, 1953)

Infectious RNA isolated from one strain of TMV by the method of Gierer & Schramm (1956) has been studied quantitatively by McLaren & Takahashi (1957). The quantum yield for inactivation at 2537 A was found to be 3×10^{-4} or about 6 times greater than for the intact virus, the reaction being first-order as for the parent virus.

It would obviously be of considerable value to compare the quantum yields for different strains of TMV and infectious RNA from each of these strains, as well as reconstituted strains and combinations of proteins and RNA from different strains. Some semi-quantitative re-

sults along these lines have already been reported by Siegel et al., (1956) and are illustrated in Fig. 12 for two different TMV strains designated by the authors as U1 and U2 for which, as can be seen from the figure, there is a 5½-fold difference in sensitivity. The two samples of infectious RNA from each virus are, however, inactivated at the same rate. But the reconstituted virus U1 is more than 5 times as sensitive to irradiation as the orginal; however since the activity of the reconstituted virus was considerably lower than that of the intact virus, it is difficult to draw any definite conclusions regarding this observation, except that one can agree with the conclusions of the authors that the sensitivity of a given strain may be dependant on the nature of the binding between the nucleic acid and protein moities. In support of this is their observation that strain U2 is more sensitive to heat denaturation than U1, while the nucleic acid of the former is also more readily released by the heat-detergent method of Fraenkel-Conrat & Williams (1956). However it is necessary to emphasize that the differences in sensitivity between the TMV strains and the RNA isolated from these strains is difficult to assess properly in view of the fact that *all* preparations were irradiated at the same concentration so that the absorption of the RNA samples was much higher than for the virus samples. It is unfortunate that quantum yields were not measured.

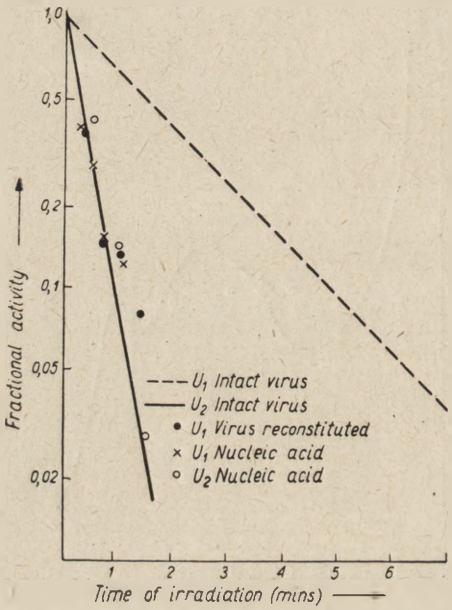


Fig. 12. The ultraviolet sensitivity of TMV strains U_1 and U_2, the infectious samples of RNA from these strains, and a sample of reconstituted U_1 strain (Siegel, Wildman & Ginoza, 1956)

More fruitful results along the above lines will undoubtedly be possible now using the newly-published method of Fraenkel-Conrat & Singer (1957) for the preparation of viruses exhibiting high activity with nucleic acids from different TMV strains.

The few observations which do exist indicate that doses sufficient to completely inactivate viruses result in only very minor modifications to the physico-chemical properties of the molecules. The inactivation of Rous sarcoma is accompanied by a 12% decrease in absorption spectrum

(Claude & Rothen, 1940). Stanley (1936) irradiated TMV to the point where infectivity was completely destroyed and found that the virus could still be crystallized from solution, the only difference being that the crystals were slightly opalescent. No marked change in molecular weight was observed, but the homogeneity of the preparation was reduced. The X-ray diffraction pattern, isoelectric point and optical rotation were unaltered. An increase in turbidity of the irradiated solution was observed but this was subsequently shown to be due to heat denaturation (resulting from the heating effect of the unfiltered high pressure mercury arc used) since irradiation at room temperature produces no such effect (Oster & McLaren, 1950). The latter authors, irradiating TMV with a filtered resonance lamp to 98% inactivation, confirmed the findings of Stanley. They also observed that the appearance of the virus under the electron microscope was unchanged (cf. Kleczkowski, 1954) and that no free nucleic acid made its appearance in the supernatant fluid following sedimentation in the ultracentrifuge.

Dulbecco (1950), who first observed the phenomenon of photoreactivation in bacteriophage, suggested that irradiated viruses may undergo two kinds of injury which he classed as photoreactiveable and non-photoreactiveable (Dulbecco, 1955). These stages of inactivation have been observed also for Rhizobium bacteriophage and for some plant viruses, but not for TMV (Bawden & Kleczkowski, 1953) and suggest some correlation between photoreactivibility and nucleic acid content, since the RNA content of TMV is only about one-third that of the other viruses. A similar correlation has been observed in the case of phages T_2, T_4 and T_6 (Luria, Williams & Backus, 1951). This cannot, however, be the full explanation since Fluke (1951) has shown that the action spectra for producing reactiveable and non-reactiveable phage each contain a minor component at 2800 Å undoubtedly due to protein.

Photochemically produced vaccines

Since Stanley's (1936) observation that ultraviolet inactivated TMV retains the serological properties of the original TMV, the separation of infectivity from the antigenic properties of viruses by radiation has been recognized as a general phenomenon and has been taken advantage of by a number of workers for the preparation of vaccines. The earlier literature on this subject is summarized by Levinson, Milzer, Shaugnessy, Neal & Oppenheimer (1944, 1945). The principle in all cases has been the same, viz. to destroy infectivity wi-

thout too much damage to the protein component of the virus so that as much of the original antigenicity as possible is retained. In some instances there is only a small mangin between the irradiation dose required to inactivate the virus and that which will destroy its antigenicity, so that careful control of conditions is an important prerequisite.

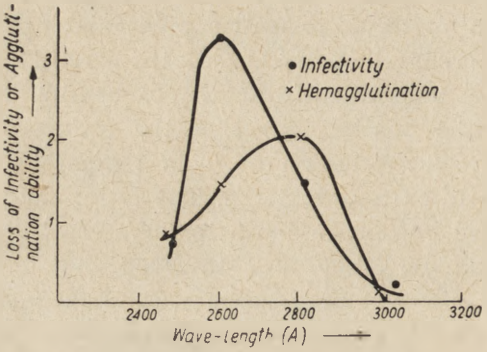


Fig. 13. Action spectra for influenza virus (Tamm & Fluke, 1950)

The theoretical basis of these procedures is clearly illustrated by the action spectra for influenza virus with respect to infectivity and hemagglutination (Fig. 13) as well as the dose-effect curves which are of the so-called „multiple-hit" type for agglutination (Fig. 14) and logarithmic for infectivity (Tamm & Fluke, 1951).

By very short exposures of continuously flowing 1 mm. thick films of virus solution to irradiation from a specially developed source with appreciable output over the entire ultraviolet range, including that below 2000 Å, Levinson et al. (1944, 1945) produced rabies and St. Louis encephalitis vaccines as well as poliomyelitis vaccine (Milzer, Oppenheimer & Levinson, 1944, 1945) and lymphocytic choriomeningitis vaccine (Milzer & Levinson 1949).

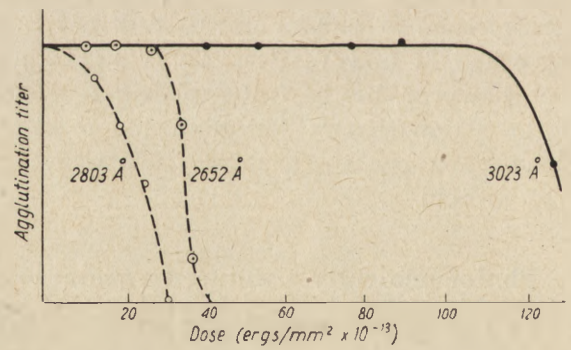


Fig. 14. Dose-effect curve for influenza virus (Tamm & Fluke, 1951)

It was subsequently shown by Habel & Sockrider (1947) that it was not so much the characteristics of the source which are of importance in this technique as the technique of irradiating thin films, and that an ordinary germicidal lamp was equally effective if allowance is made for its lower intensity output.

Similar procedures have been used by McKinstry & Reading (1944) for SK-mosaic poliomyelities vaccine, and Andrewes, Elford & Niven (1948) for vaccinia virus which, however, while reported to immunize mice against ectromelia, offered little protection to rabbits and guinea-pigs against vaccinia, a finding which Collier, McLean & Vallet (1955) ascribe to the fact that immumnizing doses were administered intraperitoneally rather than subcutaneously, that the interval between doses was too short and that excessive irradiation occurred as a result of the irradiation technique used.

Collier at al. (1955) have used the Habel & Sockrider (1947) irradiation device to produce ultraviolet inactivated vaccinia virus which produced good immunity responses in rabbits and monkeys and suggest that irradiated virus may also be useful in special cases where vaccination is normally contraindicated.

More recently the American authors have extended their original procedure to produce a poliomyelities vaccine which is claimed to compare favourably with formalin-inactivated vaccines. The principle used involved a combination of irradiation, followed by warming of the irradiated virus to 37—40°C (Shaugnessy, Wolf, Janota, Neal, Oppenheimer, Milzer, Naftulin & Morrissey, 1957). In view of the generally increased susceptibility of enzyme activity and virus infectivity to heat, after a period of irradiation sufficient to only partially destroy activity (see above), this principle will undoubtedly find wider application.

Ultraviolet irradiation has also been succesfully applied to the freeing of plasma products (intended for clinical use) from the viruses causing hepatitis and jaundice (McCall, Gordon, Bloom, Hyndman, Taylor & Anderson, 1957).

Indirect effects of radiation

Up to the present we have been dealing mainly with effects resulting from the direct absorption of radiation by the constituents under study. However since nucleic acids, nucleotides and nucleoproteins exist in living cells usually in combination with, or in proximity to, other cellular components, there exist various possibilities that absorption of light by other cell components may provoke changes in nucleotides or polynucleotides through energy transfer (already discussed above), through the action of free radicals, photodynamic action, etc.

In the case of enzymes attention has been drawn to the possible relative importance of sensitized photo-oxidation as compared to direct

irradiation effects, both by visible as well as ultraviolet light, in irradiation effects on living organisms (Shugar, 1951c).

The viscosity of solutions of DNA is decreased by irradiation with visible light in the presence of photodynamically active compounds such as dyes and carcinogens (Koffler & Markert, 1951), although the conclusion that depolymerization occurs is probably unjustified. The same effect was shown to result from irradiation with ultraviolet light, due allowance being made for the effect of absorption in this case by the DNA itself. However the quantum yield for this reaction must have been very low indeed. Zamenhof, Leidy, Hahn & Alexander (1956) irradiated samples of DNA, exhibiting transforming activity, in the presence of acriflavine with visible light from a 150-watt lamp at 1.5 cms. for 1 hour. No loss of transforming activity was noted under these conditions. Acriflavine was not used by Koffler & Markert; but one of the dyes used by them, eosin, required 7.3 hours irradiation (under conditions which we estimate to be similar to those used by Zamenhof et al.) to cause a 20% decrease in viscocity of DNA. For methylene blue the corresponding period was 55 hours.

Alexander & Fox (1954) have studied the photodynamic degradation of DNA and polymethylacrylic acid by visible light in the presence of rose bengal and acriflavine. Because of its greater sensitivity polymethylacrylic acid was examined in greater detail and the authors state that the process involves the breaking of carbon-carbon bonds. Since the reaction could be inhibited by the addition of chemicals which normally counteract the degradation produced by X-irradiation, it was concluded that the photodynamic reaction is the result of the formation of HO_2 radicals formed by the action of dissolved O_2 with the activated dye molecule as follows:

$$D \xrightarrow{h\nu} D^*$$

$$D^* + O_2 \rightarrow D^- + O_2^-$$

$$O_2^- + H_2O \rightarrow OH^- + HO_2\cdot$$

While such a mechanism may be a conceivable one, it is certainly not the only one; no detailed experimental data for DNA are presented.

The effects of photochemically produced radicals on DNA have been extensively studied by Butler & Conway (1953) and found to quantitatively resemble the action of X-rays. The DNA was irradiated in the presence of H_2O_2 at wavelengths below 3100 Å so that hydrogen peroxide is dissociated with almost unit efficiency:

$$H_2O_2 \xrightarrow{h\nu} 2\,OH\cdot$$

These photochemically produced hydroxyl radicals are much more effective in degrading nucleotide chains than ultraviolet alone, the reactions brought about including deamination and dehydroxylation of the bases, ring destruction, chain breakage, oxidation of the carbohydrate moieties (Butler, 1954, 1955).

Alper (1954), on the other hand, reports that phage exposed to ultraviolet plus H_2O_2 is inactivated only to the same extent as it would be by each acting alone, the conclusion being that OH radicals are without effect on the phage.

Considerably more attention has been devoted to the photosensitized inactivation of viruses. Herzberg (1953) observed that thiazine, acridine and thioxanthone dyes sensitize vaccinia virus to visible light, methylene blue and thiopyronine acting most rapidly at a dilution of 10^{-6}. Galston & Baker (1949) found riboflavin to be quite active as a photosensitizer of T_{2r} phage to visible light; and Galston (1950) reports that riboflavin is also effective against phage T_{6r}, the inactivation curve being of the "multiple-hit" type as compared to the first-order reaction normally found for such processes. The activity of riboflavin against viruses is furtheremore not general, as shown by the results of Welsh & Adams (1954), below.

Oster & McLaren (1950) showed that TMV is inactivated according to a first-order reaction by irradiation with blue light in the presence of acriflavine and that photooxidation was involved since removal of oxygen from the system resulted in a smaller loss of activity. Evidence was presented also to show that binding of the dye to the virus was involved in the reaction; in the presence of salt, which reduces the extent of binding of the dye, the loss in activity was considerably reduced.

Such photosensitization mechanisms also have their counterpart *in vivo* (Errera, 1953; see Blum, 1941, 1954).

Several bacteriophages have been found to be inactivated by exposure to visible light with an action spectrum exhibiting a maximum in the near ultraviolet region. The cause of this is not clear, but could be due to the presence in the virus molecule of some pigment (Wahl & Latarjet, 1946, 1947). This effect has been verified for a number of phages by Welsh & Adams (1954) but is very small compared to the effect obtained in the presence of dyes, of the order of 1% of the rate with methylene blue.

A very comprehensive study of photodynamic inactivation of phages is that of Welsh & Adams (1954) who investigated 14 different strains from several serological groups. Neither riboflavin nor eosin, which are known effective sensitizers for other systems (see above)

were effective but methylene blue was active in all cases. Except for a short initial lag period, inactivation was logarithmic for all strains examined. From the influence of dye concentration and pH on the reaction kinetics it appeared that the rate-determining factor was the amount of dye absorbed to the phage particles. Of particular interest was the fact that kinetic studies confirmed the earlier finding of Burnet (1953) according to which serologically related phages exhibit similar susceptibility, with marked differences from one serological group to another. In the case of T_2 phage, destruction of host-killing property occured at only one-third the rate of loss of plaque-forming ability. Attempts to demonstrate photoreactivation and multiplicity reactivation were unsuccesful, in agreement with Galston's (1950) finding for T_{6r} phage, and showing that the mechanism involved is quite different from ultraviolet irradiation.

The relation between the chemical structure and photodynamic activity of various dyes against the T-group phages has been extensively investigated by Yamamoto (1956). Thiazine, oxazine and acridine dyes were found to be very active and the folowing features were commmon to all of them: (a) active dyes are basic hetero-cyclic compounds possessing two N atoms; (b) the distance between these two N atoms is almost equal in the various dyes and a conjugated double bond system lies between them; (c) all of the dyes possess an ionic affinity for nucleic acids. It was concluded that the most important factor is this affinity between dye and phage (cf. Oster & McLaren, 1950) and that excited oxygen is involved only in an intermediate step in the process.

Preparative photochemistry

The practical and potential applications of photochemistry to the preparation of new compounds have been extensively discussed by Schenck (1952) and Masson, Boekelheide & Noyes (1956). While photochemical methods are, except in some special cases, limited in scope in that it is usually not possible to predict the course of the reaction, nor is it feasible to prepare large quantities of the photoproduct, yet they may prove to be of some interest in the synthesis of new pyrimidine derivatives, particularly in those cases where the reaction is reversible.

The photochemical preparation of 6-hydroxy-1,3-dimethylhydrouracil (Moore & Thomson, 1955, 1957) is a good example, in that it was this work which led to the development of a chemical synthesis

(Wang et al., 1956) of this compound. It is clear from what has been presented above regarding the behaviour of uracil derivatives that a wide variety of dihydrouracil derivatives may be prepared photochemically, although it remains to be shown whether these compounds possess any biological significance. Some preliminary observations of Moore & Thomson (1956) also suggest the possibility of preparing some 5,6 alkyl-substituted uracil derivatives. A similar situation applies to cytosine derivatives, although the much greater instability of these compounds is likely to render this task difficult.

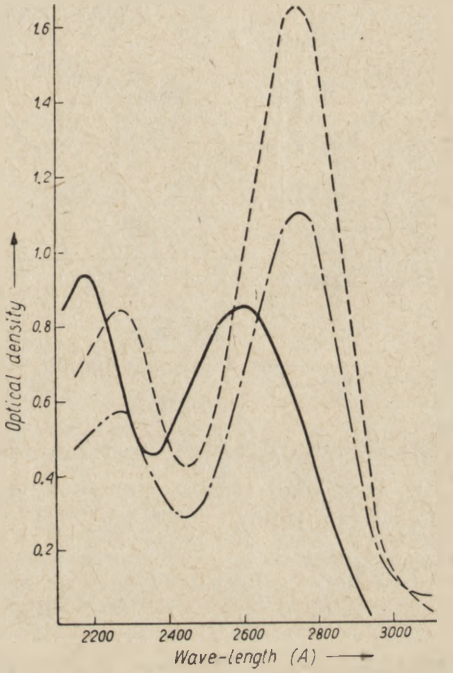
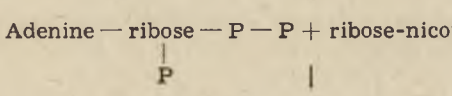


Fig. 15. 2-Ethoxyuracil in 0.02 M phosphate buffer pH 7.2, ——— before irradiation, — — — — after 150 min photolysis (2537 A), -.-.-.- after 140 min at 85°C

However, as pointed out above in the section on pyrimidines, a number of ethoxy derivatives of uracil and cytosine give photoproducts which are much more stable even than the photoproduct of 1,3-dimethyluracil and work is now in progress in this laboratory on the isolation and identification of these products which, from their absorption spectra, do not correspond to any other known pyrimidine derivatives (Figs. 15 & 16).

The unique course of the reaction leading to the addition of a water molecule to the 5 : 6 double bond of so many pyrimidines and their nucleosides and nucleotides suggest also the possibility of using this as a means of labelling these molecules with deuterium or tritium in the 5 position of the pyrimidine ring.

Irradiation of TPN results in the formation of 7-minute hydrolyzable phosphate, all of which is found in a compound in which the ratio adenine: total P : ribose : 7-minute- P : : 1.1 : 3 : 0.9 : 1.2, corresponding to a compound resulting from the cleavage of TPN as follows:



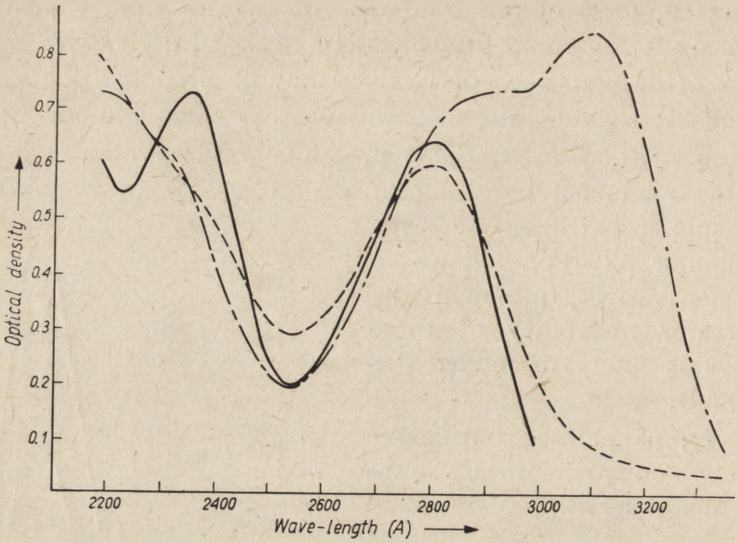


Fig. 16. 2-Methoxycytosine in 0.02 M phosphate buffer pH 7.2
——— before irradiation, -.-.-.-. after 150 min phototysis (2537 A),
— — — — — after 40 min at 85°C

The structure of this compound has been tentatively verified by incubation with potato phosphorylase and potato 5'-nucleotidase (Seraydarian, 1955).

REFERENCES

1. Alexander P. & Fox M. — 1st Intern. Photobiol. Cong., Amsterdam 1954.
2. Alper T. — 1st Intern. Photobiol. Cong., Amsterdam 1954.
3. Andersen E. H. — J. Bact. **61**, 389, 1951.
4. Andersen W., Hayes D., Michelson A. & Todd A. R. — J. Chem. Soc., 1882. 1954.
5. Andrews C. H., Elford W. J. & Niven J. S. F. — Brit. J. Exp. Path. **29**, 329, 1948.
6. Atwood K. C. & Norman A. — Proc. Natl. Acad. Sci, **35**, 696, 1949.
7. Bannister T. T. — Arch. Biochem. Biophys., **49**, 222, 1954.
8. Bass L. W. — J. Am. Chem. Soc., **46**, 190, 1924.
9. Batt R. D., Martin J. K., Ploeser J. McT. & Murray J. — J. Am. Chem. Soc., **76**, 3663, 1954.
10. Bawden F. C. & Kleczkowski A. — J. Gen. Microbiol., **8**, 145, 1953.
11. Bawden F. C. & Kleczkowski A. — Nature, **169**, 90, 1952.
12. Bawden F. C. & Kleczkwski A. — J. Gen. Microbiol., **13**, 370, 1955.
13. Bendich A. & Clements G. C. — Biochim. Biophys. Acta, **12**, ,462, 1953.
14. Benzer S. & al. — in *Viruses*, ed. M. Delbruck, Cal. Inst. Technol., Pasadena, 1950.

15. Berson J. A. & Brown E. — J. Am. Chem. Soc., **77**, 447, 1955.
16. Blum H. — *Photodynamic Action & Diseases Caused by Light*, Reinhold, N. Y. 1941.
17. Blum H. — *In Biophysical Research Methods*, ed. F. M. Uber, Interscience, N. Y. 1950.
18. Blum H. — 1st Intern. Photobiol. Cong., Amsterdam 1954.
19. Bowen E. — *The Chemical aspects of Light*, Clarendon Press, Oxford 1946.
20. Brandt C. L. & Giese A. C. — J. Gen. Physiol., **39**, 735, 1956.
21. Broser W. & Lautsch W. — Z. Naturforsch. **11b**, 453, 1956.
22. Brown D. M., Cohran W., Medlin E. H. & Varadarajan S. — J. Chem. Soc., 4873, 1956.
23. Brown D. M., Todd A. R. & Varadarajan S. — J. Chem. Soc., 2388, 1956.
24. Bucher T. & Kaspers J. — Biochim. Biophys. Acta **1**, 21, 1947.
25. Bucher T. — Ad. Enzymol., **14**, 1, 1953.
26. Burnet F. M. — J. Path. Bact. **37**, 179, 1933.
27. Butler J. A. V. — 1st Intern. Photobiol. Cong., Amsterdam 1954.
28. Butler J. A. V. — Experientia **11**, 289, 1955.
29. Butler J. A. V. & Conway B. E. — Proc. Roy. Soc. **141**, 562, 1953.
30. Buttolph L. J. — in *Radiation Biology*, Vol. II, McGraw-Hill, N. Y., 1955
31. Buzzel A. — Arch. Biochem. Biophys., **62**, 97, 1956.
32. Calcutt G. — Nature **166**, 443, 1950.
32a. Calvert J. G. & Rechen H. J. L. — J. Am. Chem. Soc., **74**, 2101, 1952.
33. Canzanelli A., Guild R. & Rapport D. — Am. J. Physiol., **167**, 364, 1951.
34. Carter C. E. — J. Am. Chem. Soc., **72**, 1835, 1950.
35. Christensen E. & Giese A. C. — Arch. Biochem. Biophys., **51**, 208, 1954.
36. Clarke V. M., Todd A. R. & Zussman J. — J. Chem. Soc., 2952, 1951.
37 Claude A. & Rothen A. — J. Exp. Med., **71**, 619, 1940.
38. Collier L. H., McLean D. & Vallet H. — J. Hygiene **53**, 513, 1955.
39. deCourcy S. J., Ely J. O. & Ross M. H. — Nature **172**, 119, 1953
40. Devreux S., Johannson M. & Errera M. — Bull. soc. chim. biol. **33**, 800, 1951.
41. Duggan D. E., Bowman R. L., Brodie B. B. & Udenfriend S. — Arch. Biochem. Biophys. **68**, 1, 1957.
42. Dulbecco R. — J.Bact., **59**, 329, 1950.
43. Dulbecco R. — in *Radiation Biology*, vol. II, ed. A. Hollaender, McGraw-Hill, N. Y. 1955.
44. Durand M. C. & Thomas R. — Biochim. Biophys. Acta **12**, 416, 1953.
45. Eckart W. — Strahlentherapie **94**, 60, 1954.
46. Ellis C., Wells A. A. & Heyroth F. F. — *The Chemical action of Ultraviolet Rays*, Reinhold Pub. Corp., New. York 1941.
47. Errera M. — Biochim. Biophys. Acta **8**, 30, 1952a.
48. Errera M. — Biochim. Biophys. Acta **8**, 115, 1952b.
49. Errera M. — Ann. Soc. Royale Sc. Med. et Natur. de Bruxelles, **5**, 65, 1952c.
50. Errera M. — Progress in Biophysics **3**, 88, 1955.
51. Fellig J. — Science **119**, 129, 1953.
52. Fluke D. J. — Phys. Rev. **82**, 302, 1951.

53. Fluke D. J. — quoted in Pollard 1953.
54. Fluke D. J. & Pollard E. C. — Science **110**, 274, 1949.
55. Fogh J. — Proc. Soc. Exp. Biol. Med. **89**, 464, 1955.
56. Forbes G. S. & Leighton W. G. — J. Am. Chem. Soc., **52**, 3139, 1930.
57. Fox J. J. & Shugar D. — Biochim. Biophys Acta **9**, 369, 1952.
58. Fraenkel-Conrat H. & Singer B. — Biochim. Biophys. Acta, **24**, 540, 1957.
59. Franklin R. M., Friedman M. & Setlow R. B. — Arch. Biochem. Biophys., **41**, 159, 1953.
60. Galston A. W., & Baker R. S. — Science **109**, 485, 1949.
61. Galston A. W. — Science **111**, 619, 1950.
61a. Garay K. & Guba F. — Acta Physiol. Acad. Sci. Hungar. **5**, 393, 1954.
62. Gierer A. & Schramm G. — Nature **177**, 702, 1956.
63. Giese A. C. — Anat. Rec. **99**, 116, 1947.
64. Giese A. C. — Physiol. Rev. **30**, 431, 1950.
65. Giese A. C. — Physiol. Zool., **26**, 1, 1953.
66. Grunberg-Manago M., Ortiz P. J. & Ochoa S. — Science **122**, 907, 1955.
67. Habel K. & Sockrider B. T. — J. Immunol. **56**, 273, 1947.
68. Henoh M. A. — Dokl. Akad. Nauk. USSR — **104**, 746, 1955.
69. Hershey A. D. & Chase M. — J. Gen. Physiol. **36**, 39, 1952.
70. Herzberg K. — Z. Immunitats. **80**, 507, 1933.
71. Heyroth F. F. & Loofbourow J. R. — J. Am. Chem. Soc. **53**, 3441, 1931.
72. Heyroth F. F. & Loofbourow J. R. — cited in Ellis et al., 1941.
73. Hollaender A., Greenstein J. P. & Jenrette W. V. — J. Nat. Canc. Inst. **2**, 23, 1941.
74. Hollaender A. & Oliphant J. W. — J. Bact., **48**, 447, 1944.
75. Hollaender A. & Zelle M. R. — 1st Intern. Photobiol. Cong., Amsterdam 1954.
76. Holtz P. — Arch. exptl. Path. Pharmakol., **182**, 141, 1936.
77. Jacobson W. & Simpson D. E. — Biochem. J. **40**, 3, 9, 1946.
78. Johnson T. B. & Johns C. O. — J. Am. Chem. Soc. **36**, 545, 1914.
79. Johnson T. B., Baudisch. O., Hoffmann A. — J. Am. Chem. Soc., **54**, 1106, 1932.
79a. Kaplan R. W. — Naturwissenschaften **42**, 184, 1955a.
79b. Kaplan R. W. — Naturwissenschaften **42**, 466, 1955b.
80. Karreman G. & Steele R. H. — Biochim. Biophys. Acta **25**, 280, 1957.
80a. Kita H., Maeda H., Hanazaki B., Shimizu F. & Fujita H. — Bull Tokyo Med. Dent. Univ., Nr 1, 37, 1954.
81. Kleczkowski A. — Biochem. J.. **56**, 345, 1954.
82. Kleczkowski J. & Kleczkowski A. — J. Gen. Microbiol., **8**, 135, 1953.
83. Koffler H. & Market I. L. — Proc. Soc. Exp. Biol. Med., **76**, 90, 1951.
84. Kurnick N. B. — Arch. Biochem., **29**, 41, 1950a.
85. Kurnick N. B. — Exp. Cell. Res., **1**, 151, 1950b.
86. Laurent T. C., Wertheim E. M. — Acta Chem. Scand., **6**, 678, 1952.
87. Laurent T. C. — J. Am. Chem. Soc., **78**, 1875, 1956.
88. Lea D. E. — *Actions of Radiations on Living Celis*, Cambridge U. Press, 1955.
89. Levinson A. B., Milzer C. D., Shaugnessy O. M., Neal V. D. & Oppenheimer F. — J. Am. Med. Assoc. **125**, 531, 1944.

90. Levinson A. A., Milzer C.D., Shaugnessy O. M., Neal V. D. & Oppenheimer F. — J. Immunol. **50**, 317, 1945.
91. Loofbourow J. R. — J. Chem. Soc., 1275, 1940.
92. Loofbourow J. R. — Growth Symp., **12**, suppl., 77, 1948.
93. Luria S. E. — Proc. Nat. Acad. Sci. U. S., **33**, 253, 1947.
94. Luria S. E. — *General Virology*, John Wiley, N. Y. 1953.
95. Luria S. E. — *Radiation Biology*, II, Chapt. 9, McGraw-Hill, New York. 1955.
96. Luria S. E., Williams R. C. & Backen R. C. — J. Bact. **61**, 179, 1951.
97. Magee J. L., Kamen M. D. & Platzman R. L. — *Basic Mechanisms* in *Radiobiology*, N. R. C. Pub. 305, 1953.
98. Masson C. R., Boekelheide V. & Noyes W. A. — *Techniques of Organic Chemistry*, vol. II, Interscience, New. York 1956.
99. McCall K. B., Gordon F. H. Bloom F. C., Hyndman L. A., Taylor H. L. & Anderson H. D. — J. Am. Pharm. Assoc., **46**, 295, 1957.
100. McKinstry D. W. & Reading E. H. — J. Franklin Inst. **237**, 71, 1944.
101. McLaren A. D. — Ad. Enzymol., 9, 75, 1949.
102. McLaren A. D. — Enzymologia **18**, 81, 1957.
103. McLaren A. D. & Takahashi W. N. — Radiation Research **6**, 532, 1957.
104. Michelson A. M & Todd A. R. — J. Chem. Soc., 816, 1955.
105. Milzer A., Oppeheimer F. & Levinson S. O. — J. Am. Med. Assoc. **125**. 704, 1944.
106. Milzner A., Oppenheimer F. & Levinson S. O. — J. Immunol., **50**, 331, 1945.
107. Milzer A. & Levinson S. O. — J. Infect. Diseases **85**, 251, 1949.
108. Mitchell H. K. — cited in Rapport et al., 1955.
109. Moore A. M. & Thomson C. H. — Science **122**, 594, 1955.
110. Moore A. M. & Thomson C. H. — 4th Intern. Conf. Radiobiol., Cambridge, Oliver & Boyd, Edinburgh, 1956.
111. Moore A. M. & Thomson C. H. — Can. J. Chem., **35**, 163, 1957.
112. Opatowski I. — Genetics **35**, 56, 1950.
113. Opatowski I. & Christiansen A. M. — Bull. Math. Biophys., **12**, 19, 1950.
114. Oster G. & McLaren A. D. — J. Gen. Physiol. **33**, 215, 1950.
115. Parker A. C. — Proc. Roy. Soc. A **220**, 104, 1953.
116. Pauling L. *Nature of the chemical bond*, Cornell University Press, 1948.
117. Peel G. N. — J. Radiol., **12**, 99, 1939.
118. Petuely F. & Meixner N. — Chem. Ber., **86**, 1255, 1953.
119. Pitts J. N., Margerum J. D., Taylor R. P. & Brim W. — J. Am. Chem. Soc. **77**, 5499, 1955.
120. Pollard E. C. — *The physis of viruses*, Academic Press New York, 1953.
121. Rapport D. & Canzanelli A. — Science **112**, 469, 1950.
122. Reddi K. — Biochim. Biophys. Acta **27**, 528, 1957.
123. Rice W. E. — Science **115**, 92, 1952.
124. Runnstrom J., Lennerstrand A. & Borei H. — Biochem. Z. **271**, 15, 1934.
125. Rutter H. A., Gustafson L. O. & Batt W. G. — J. Franklin Inst., **260**, 329, 1955.
126. Santer E. — Z. angew. Phys. **9**, 105, 1957.
127. Schencke G. O. — Angew. Chem. **64**, 12, 1952.

128. Scott J. F. & Sinsheimer R. L. — *Radiation Biology*, vol. II ed. A. Hollaender, McGraw-Hil, N. Y. 1955.
129. Siegel A. & Wildman S. G. — Phytopath. **44**, 277, 1954.
130. Siegel A., Wildman S. G., & Ginoza W. — Nature, **178**, 1117, 1956.
131. Seraydarian M. W., Canzanelli A. & Rapport D. — Am. J. Physiol. **172**, 42, 1953.
132. Seraydarian M. W., Cohen A. I. & Sable H. Z. — Am. J. Physiol., **177**, 150, 1954.
133. Seraydarian M. W. — Am. J. Physiol. **181**, 291, 1955.
134. Seraydarian M. W. — Biochim. Biophys. Acta **19**, 168, 1956.
135. Setlow R. & Doyle B. — Biochim. Biophys. Acta **12**, 508, 1953.
136. Setlow R. & Doyle B. — Biochim. Biophys. Acta **15**, 117, 1954.
137. Setlow R. & Doyle B. — Biochim. Biophys. Acta **24**, 27, 1957.
138. Shaughnessy H. J., Wolf A. M., Janota M., Neal J., Oppenheimer F., Milzer A., Naftulin H. & Morrissey R A. — Proc. Soc. Exp. Biol. Med. **95**, 251, 1957.
139. Shore V. G. & Pardee A. B. — Arch. Biochem. Biophys., **60**, 100, 1956a.
140. Shore V. G. & Pardee A. B. — Arch. Biochem. Biophys., **62**, 335, 1956b.
141. Shugar D. — Experientia **7**, 26, 1951a.
142. Shugar D. — Biochim. Biophys. Acta **6**, 548, 1951b.
143. Shugar D. — Bull. soc. chim. biol. **33**, 710, 1951c.
144. Shugar D. & Fox J. J. — Biochim. Biophys. Acta **9**, 199, 1952.
145. Shugar D. & Wierzchowski K. L. — Biochim. Biophys. Acta **23**, 657, 1957a.
146. Shugar D. & Wierzchowski K. L. — Intern. Symp. Macromol. Chem., Prague, J. Polymer Sci. (in press), 1957b.
147. Shugar D. & Adamiec A. — work in progress, 1957.
148. Sinsheimer R. L. & Hastings R. — Science **110**, 525, 1949.
149. Sinsheimer R. L. — Radiation Research **1**, 505, 1954.
150. Sinsheimer R. L. — Radiation Research **6**, 121. 1957.
151. Stanley W. M. — Science **83**, 626, 1936.
152. Stimson M. M. & Loofbourow J. R. — J. Am. Chem. Soc., **63**, 1827, 1941.
153. Stimson M. M. & Reuter M. A. — J. Am. Chem. Soc. **65**, 697, 1941.
154. Stimson M. M. — J. Am. Chem. Soc. **64**, 1604, 1942.
155. Swenson P. A. & Giese A. C. —J. Cell. Comp. Physiol. **36**, 369, 1950.
156. Tamm C., Hodes M. E. & Chargraff E. — J. Biol. Chem. **195**, 49, 1952.
157. Tamm I. & Fluke D. — J. Bact. **59**, 449, 1950.
158. Tanada T. & Hendricks S. B. — Am. J. Botany, **40**, 634, 1953.
159. Timofeeff-Ressovsky N. K. & Zimmer K. G. — *Das trefferprinzip in der Biologie*, Hirzel, Leipzig, 1947.
160. Uber F. M. & Verbrugge F. — J. Biol. Chem. **134**, 273, 1940.
161. Uber F. M. — Nature **147**, 148, 1941.
162. Vandendriesche L. — C. R. Trav. Lab. Carlsberg, Ser chim. **21**, 341, 1951.
163. Wahl R. & Latarjet R. — Ann. Inst. Past **72**, 284, 1956.
164. Wahl R. & Latarjet R. — Ann. Inst. Past. **73**, 957, 1947.

165. Wang S. Y., Apicella M. & Stone B. R. — J. Am. Chem. Soc., **78**, 4180, 1956.
166. Wells P. H. — Science **124**, 31, 1956.
167. Welsh J. N. & Adams M. H. — J. Bact. **68**, 122, 1954.
168. Welsch M. M. & Minon A. — C. R. soc. biol. **149**, 1309, 1955.
169. Wiberg K. B. — Chem. Rev. **55**, 713, 1955.
170. Wierzchowski K. L. & Shugar D. — Annual Congress „Chemische Gesellschaft DDR", Leipzig, 1956.
171. Wierzchowski K. L. & Shugar D. — Biochim. Biophys. Acta, **25**, 355, 1957.
172. Wyss O. — 1st Intern. Photobiol. Cong., Amsterdam 1954.
173. Yamamoto N. — Virus (Osaka) **6**, 510, 522, 531, 1956.
174. Zamenhof S., Leidy G., Hahn E. & Alexander H. E. — J. Bact. **72**, 1, 1956.
175. Zelle M. R. & Hollaender A. — J. Bact. **68**, 210, 1954.
176. Zill L. P. — Fed. Proc. **16**, 276, 1957.

ADDENDUM

Since submission of the above review Botner-by & Balazs (Radiation Research 6, 302, 1957) have reported the results of a study of the irradiation of alkaline solutions of glucose with X-rays; the products of irradiation were found to be identical with those resulting from ultraviolet irradiation, thus confirming the interpretation presented above (see page 263) of the experments of Rice (1952) and Laurent & Wertheim (1952).

A systematic investigation of the photochemistry of purines has been initiated by Kland & Johnson (J. Am. Chem. Soc. 79, 6187, 1957). An initial lag period was found to prevail for those compounds hitherto examined and including adenine, guanine, xanthine, hypoxanthine and uric acid; the extent of the lag period varying for the different purines. At high irradiation intensities dilute solutions of all the compounds (for adenine this occurred only during the induction period under oxygen) exhibited a decomposition rate proportional to the square of the irradiation time. Apparently no simple rate law prevailed under any of the conditions of irradiation. The results have been used to divide purines into classes as regards susceptibility to irradiation under oxygen or nitrogen, The influence of the former was emphasized by the fact that decomposition of adenine under oxygen was complete as compared to only 10% decomposition under nitrogen, The isolation of hypoxanthine from irradiated adenine under oxygen was taken to indicate that photolysis proceeds predominantly by way of oxidative breakdown involving attack at the amino group, although the considerably lower rate under nitrogen suggested also some hydrolytic action. However the evidence for these mechanisms is hardly conclusive since the amounts of hypoxanthine isolated were negligibly small, nor was this the only photoproduct obtained. The results also point to the participation of free radicals and it is unfortunate in this respect that, although resonance lamps were used, exposure times were extremely long and no attempt was made to filter out traces of shorter wavelengths.

In a study the details of which have not yet come to hand, Moore (Can. J. Chem. 36, 281, 1958) reports the synthesis of the product of reversible photolysis of uracil (Curr. Chem. Papers. Nr. 4, 1958).

Two further examples of the reversal of the effects of ultraviolet irradiation on plants have been reported (cf. page 275). Owen (Nature **180**, 610, 1957) irradiated tobacco leaves for 4′ at **2537** A, the respiration of detached leaves being increased in this way by 20% ever a 10-hour period; a 2-hour exposure to natural light following ultraviolet irradiation was found to completely reverse the stimulatory effect of ultraviolet. Tanada (Am. J. Botany **44**, 723, 1957) examined the influence of ultraviolet on the absorption of rubidium by Mung bean roots and reports that post-treatment of irradiated roots with EDTA (versene) followed by treatment with β-mercaptoethylamine and ATP resulted in a major reversal of the effects of irradiaton.

An interesting study (Tolmach & Lerman, Radiation Research Society, Rochester, 1957; Radiation Research **7**, 454, 1957), the details of which are as yet unpublished, has been made on the selective effects of ultraviolet irradiation on biologically active DNA. Irradiation of ^{32}P-labelled undegraded pneumococcal DNA showed that the capacity of this latter to undergo incorporation is much less sensitive than its transformation capacity; in addition the sensitivities of two genetic markers were substantially different. The inactivation curves for both markers were complex, but suggestive of a one-hit process.

An extensive review of the photochemistry of viruses has been prepared by Kleczkowski (Virus Research IV, 191, 1957) and a short text on „Excited States in Chemistry & Biology" (Reid, Butterworth Scientific Pubs., London, 1957) will undoubtedly provide stimulation to many photochemists in the biological field. The subject of action spectra has also been brought up to date by Setlow (Ad. Med. Biol. Phys. **5**, 37, 1957).