ACKNOWLEDGEMENT

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SUMMARY

The results of setting experiments show that SO_3^{2-} and OH^- are the main reactive ions when the cystine disulphide cross-linkages in human hair are attacked by sulphite/bisulphite solutions in 10% EtOH at 50°.

S-Cysteine sulphonate, $RSSO_3^-$, incorporated in the protein chain, is the most important source of bisulphite-stable set at the lower pH values (4 to 8) investigated. The cross-linkages involved are formed in the reaction mixtures at 50° and also when a treated fibre is immersed slack in boiling distilled water.

The cross-linking reaction is alkali-catalysed.

The second side-chain which combines with $RSSO_3^-$ to form a bisulphite-stable cross-linkage will be discussed in a following paper.

The sulphenic acid group, RSOH, incorporated in the protein chain also gives rise to bisulphite-stable cross-linkages; its effect becomes appreciable at pH values above 8. Viewed with other evidence[6], this suggests that the cross-linkages in this case are lanthionine, RSR, bonds.

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PHOTOCHEMISTRY OF CYTOSINE NUCLEOSIDES AND NUCLEOTIDES

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The effects of ultraviolet irradiation of nucleic acids and their derivatives have received considerable attention, largely because of their widespread occurrence in living organisms, their high selective absorption in ultraviolet light, and considerable evidence pointing to them as the immediate and principal receptors of radiation resulting in a variety of biological effects. Although it is well known that purines are considerably more resistant to irradiation than pyrimidines, there is a surprising paucity of quantitative data on this subject.

In most instances the photolysis of purine and pyrimidine derivatives is accompanied by a destruction of the absorption spectrum and the formation of a wide variety

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of products. However, in 1949 SINSHEIMER AND HASTINGS[1] made the interesting observation that uridylic acid and uracil, following irradiation at 2537 A until the principal absorption maximum had completely disappeared, could be made to regain the original spectrum by acidifying to pH 1. Cytidylic acid, but not cytosine, was reported to exhibit similar behaviour. The photolysis of uridylic acid was subsequently extensively studied by SINSHEIMER[2], who showed that the reaction could also be reversed by heat at neutral pH; and it was demonstrated by RAPPORT, CANZANELLI AND SOSSEN[3] and by MITCHELL (quoted by RAPPORT *et al.*[3]) that the reconstituted substance behaved biologically like the original. Evidence presented by MOORE AND THOMPSON[4] indicated that the disappearance of the absorption maximum of 1,3-dimethyluracil upon irradiation was due to loss of the 5:6 double bond caused by the addition of a molecule of water. This was confirmed by WANG, APICELLA AND STONE[5], who showed that the photoproduct is 6-hydroxy-1,3-dimethylhydrouracil. Reversal of the effects of irradiation by acid or heat in the dark is due to removal of the water molecule, with the resultant reformation of the double bond. Studies of the forward and backward reactions in light and heavy water were in accord with these findings and indicated, indirectly, that a similar mechanism holds for the irradiation of cytosine and a number of its derivatives, particularly its nucleosides and nucleotides[6].

The fact that the photolysis of nucleosides and nucleotides of cytosine and uracil may be reversed in the dark is of obvious interest, in view of the findings that the effects of ultraviolet irradiation on microorganisms may be partially reversed by light or heat. In view of the fact that the immediate receptors of the primary radiation are most likely nucleic acids, it is quite conceivable that at least a partial answer to the phenomenon of photoreactivation in microorganisms may be found in the reversible photolysis of the pyrimidine nucleotides (unsubstituted in the 5,6 positions, see below) of the nucleic acids, and this is one of the objects of our investigations[6].

We have been studying the ultraviolet photolysis of a wide variety of pyrimidine analogues and in this publication report some of our observations on the reversible photolysis* of cytosine derivatives. We shall show elsewhere that the irradiation of some pyrimidines (*e.g.* 2-methoxyuracil), while not necessarily reversible, results, not in a rupture of the pyrimidine rings, but in the formation of new pyrimidine derivatives at neutral pH.

MATERIALS

A number of the compounds used have been previously described[7,8]. Cytidylic acid was a mixture of the *a* and *b* isomers. Desoxycytidylic acid was a gift of the California Foundation for Biochemical Research. 2-Methoxycytosine was synthesized by the method of HILBERT AND JOHNSON[9], as before. All substances were checked spectrophotometrically and/or chromatographically. Phosphate and borate buffers, 0.015 *M*, were used in the pH range 6–9; acid and alkaline pH values were obtained by appropriate dilutions of HCl and NaOH and with 0.03 *M* glycocoll buffers.

METHODS

A Thermal Syndicate mercury resonance lamp was used as a source of 2537 A radiation. Although more than 95% of the energy of this lamp is emitted at this wavelength, it was found that traces of radiation at shorter wavelengths were sufficiently active to produce additional reactions (as well as to interfere frequently with the subsequent reversibility of the reactions); an acetic acid

* A reversible photochemical reaction is here understood to be one where the effects of irradiation may be subsequently reversed in the dark.

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filter was consequently used to eliminate all wavelengths below 2400 A. For qualitative studies, and also where overnight exposures were required, a Philips germicidal lamp was employed. In quantitative studies, solutions were irradiated in spectrophotometer cuvettes of 2 to 10 mm, without stirring and with such concentrations of the substance under investigation that initial optical densities were in the neighbourhood of 0.6–0.8 ($\sim 10^{-4}$ *M*).

Light intensities were measured by uranyl oxalate actinometry[10] and were of the order of 10^{16} quanta/cm²/min. From time to time, however, it was found more convenient to check source intensities by the rate of photolysis of one of the nucleosides, which had been previously standardized against the uranyl oxalate actinometer.

When the effects of photolysis were to be reversed by heat, the solutions were heated in the original quartz cuvettes used for irradiation and spectral measurements, in a Hoepler thermostat; any losses by evaporation were made up by the addition of water.

Spectrophotometric measurements were made with a Unicam SP-500 and a Soviet SF-4 spectrophotometer, both of which were checked against known compounds. The agreement between the two instruments was excellent for wavelengths down to 2050 A.

RESULTS

From an investigation of a wide range of pyrimidine derivatives it became evident that, for reversible photolysis to take place, the number 5 and 6 positions in the pyrimidine ring must be unsubstituted (see also ref. [2]). This is, however, not a sufficient condition, for compounds such as 2-thiocytosine and 2-thiouracil fail to exhibit the phenomenon of reversibility. The electron distribution density in the pyrimidine ring is thus also of some importance.

The cytosine derivatives exhibiting reversible photolysis may be divided into two groups insofar as their photochemical behaviour (as well as the rate and extent of reversibility) is concerned: (a) cytosine and 1-methylcytosine and (b) nucleosides and nucleotides of cytosine.

The products of irradiation of compounds of group (b) are remarkably resistant to irradiation over a wide range of pH values, and, even after long periods of irradiation, more than 95% of the original absorption spectrum may be reconstituted at acid, alkali (pH $\leq$ 12) and neutral pH. Even after overnight irradiation of pyranosylcytosines, for example, more than 50% of the photoproduct can still revert to the original substance. Some idea of the rate and extent of reversibility for several compounds may be gained from an inspection of Figs. 4, 5 and 6. Following irradiation at neutral pH, reversal is most rapid upon acidification, in some instances too rapid to measure.

The products of irradiation of compounds of group (a) are, on the other hand, much more sensitive photochemically so that after lengthy periods of irradiation no evidence of reversibility at all may be observed. This undoubtedly explains why SINSHEIMER AND HASTINGS[1] failed to note any reversibility of the photolysis of cytosine.

In group (a) irradiation is accompanied by the disappearance of the spectrum over the entire range examined, whether the reaction proceeds in acid (Fig. 1), neutral[6] or alkaline (Fig. 2) medium. In unbuffered solution the disappearance of the absorption spectrum is accompanied by a small shift towards the red of the principal maximum at 2700 A, a phenomenon not observed in buffered medium at the same pH. Furthermore, in going from neutral to acid medium there is no appreciable change in quantum yield (Table I), despite the fact that there is uptake of a proton by the amino group (p*K* values for cytosine and 1-methylcytosine, 4.45 and 4.55, respectively[7, 8]). On the other hand, in acid medium the reaction is no longer reversible (Fig. 1). For 1-methylcytosine there is little change in quantum yield (Φ) from pH 2 to 14. In the case of

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cytosine enolization of the carbonyl group (pK 12.2[7]) is accompanied by an abrupt increase in Φ of almost one order of magnitude. Finally reconstitution of both these compounds in unbuffered medium (pH ~5.6) is such that the final spectrum has its principal maximum displaced about 50 A to the red of the original.

Photolysis of cytosine in alkaline medium (pH 13–14) results in a product which undergoes further modification in the dark so that the final principal maximum is about 100 A off to the violet (Fig. 2).

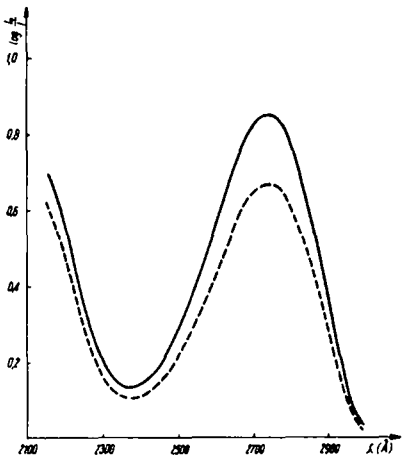

Fig. 1. Cytosine in 0.01 N HCl. ——— before irradiation; ------- following 110′ irradiation (no change after 48 h at room temp.).

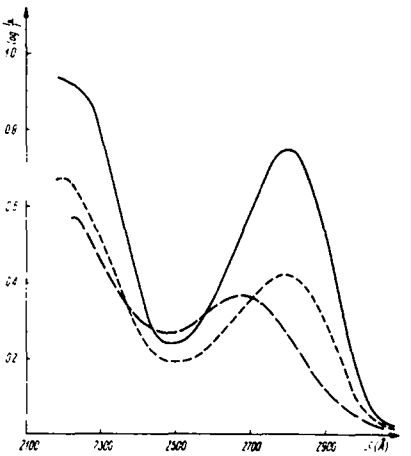

Fig. 2. Cytosine in 0.1 N NaOH. ——— before irradiation; ------- after 80′ irradiation; ----- after 15 h at room temp.

TABLE I

QUANTUM YIELDS FOR PHOTOLYSIS OF CYTOSINE DERIVATIVES AT 2537 A (MOLE/EINSTEIN × 10^3)

Compound	pH								
	1	2	H_2O*	7.1	8.9	10.5	11	13	14
Cytosine	—	1.3	1.7**	1.3**	—	—	—	6.3	8.0
1-Methylcytosine	—	1.4	2.1**	1.3**	—	—	—	2.1	—
Cytidine	—	1.6**	9.0**	10**	—	8.9**	6.8**	3.6	2.9
Cytidylic acid	1.1**	—	—	12.5**	—	9.4**	6.3**	1.4	—
Desoxycytidine	—	1.6**	9.5**	8.6**	—	8.3**	6.0**	0.5	—
Desoxycytidylic acid	—	1.5**	—	2.9**	3.0**	3.0**	3.0**	1.0	—
Pyranosyl-cytosines	1.6**	—	16**	13**	15**	11**	7.9**	1–2§	0.25–2.5§

* pH ~ 5.6. ** Reaction reversible in the dark. § Quantum yield increases during reaction.

In compounds of group (b) an entirely different behaviour is observed both as regards changes in absorption spectra upon irradiation, as well as quantum yields. Over the pH range 6–10.5, where neither the pyrimidine amino nor the carbohydrate hydroxyls are dissociated, there is a decrease in the height of the principal absorption maximum in the neighbourhood of 2700 A with simultaneous appearance of a new maximum in the region 2360–2390 A (Figs. 5 and 6, see also ref. [7]). Strictly speaking, this is not necessarily a new maximum, as may be seen from Fig. 5 (see DISCUSSION). In this pH range the quantum yield is about the same for all of these compounds, with the excep-

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tion of desoxycytidylic acid, and about one order of magnitude greater than for cytosine and 1-methylcytosine. Furthermore, the reaction can be reversed in the dark, the rate of reversal being dependent on pH and temperature.

At pH > 12 the quantum yields drop appreciably (Table I), while the reaction is no longer reversible either by acidification or heating. In this pH range photolysis is accompanied by the disappearance of the entire spectrum (Fig. 3).

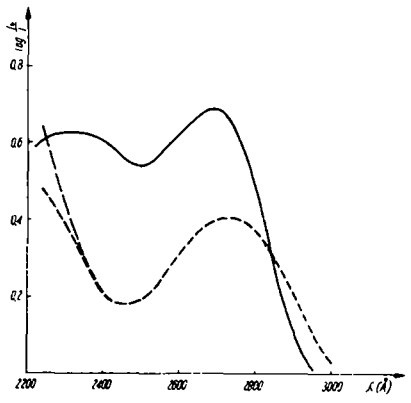

Fig. 3. Galactopyranosylcytosine in 1 *N* NaOH. —— before irradiation; ------ following 150′ irradiation; – – – – after 18 h at room temp.

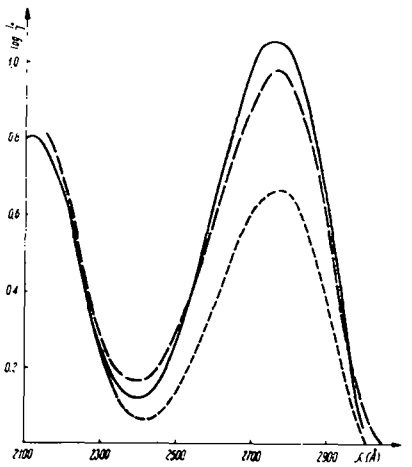

Fig. 4. Glucopyranosylcytosine in 0.1 *N* HCl. — before irradiation; ------- after 220′ irradiation; · · · · after 18 h at room temp.

In acid medium, where the amino group is dissociated, the quantum yield is one order of magnitude lower and its value is practically identical for all compounds; during irradiation the absorption maximum at 2800 A decreases, while there is no marked change in the region 2100–2300; following irradiation the photoproducts revert to the original substance (Fig. 4).

Neither in acid nor in alkaline medium does the maximum at 2360 A appear during irradiation. The height of this maximum during irradiation is not markedly dependent on pH in the range 7–10.5. However, at pH values below 7, such that there is as yet no decrease of Φ with pH, photolysis is accompanied by only a slight increase in absorption in this region. That this is only a pH effect (due, most likely to the dissociation of some group in the photoproduct, probably the amino group) is testified to by the fact that, if pyranosylcytosines are irradiated in water (pH ~5.6) and the pH after irradiation brought to 7.2, the maximum at 2360 immediately makes its appearance with the same optical density as normally results from irradiation at this pH (Fig. 5).

As already mentioned above, desoxycytidylic acid differs appreciably from the other nucleosides and nucleotides in photochemical behaviour in that Φ is considerably lower in the pH range 7–11. One further significant difference is that the maximum at 2360 A resulting from irradiation makes its appearance only on the alkaline side of pH 8, while its height is less than that for the other compounds in this group (Fig. 6). In other respects its behaviour is qualitatively similar.

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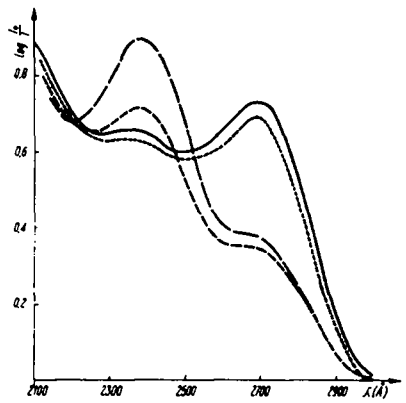

Fig. 5. Galactopyranosylcytosine in water. ——— prior to irradiation; ------ after 25′ irradiation; – – – – pH brought to 7.2 following irradiation; ····· after 18 h at room temp. pH 7.2 (time for 50% reversal about 2½ h) *or* after 3′ at 70°C.

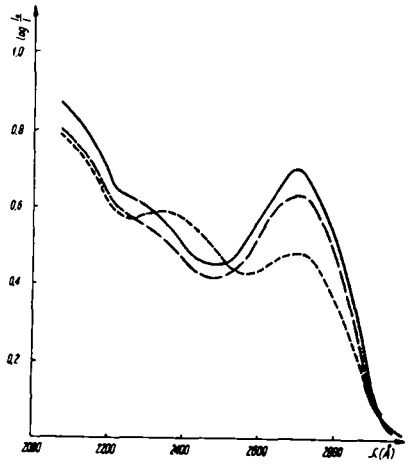

Fig. 6. Desoxycytidylic acid at pH 8.4. – – – – prior to irradiation; ------- following 145′ irradiation; – – – – – after 18 h at room temp. *or* 5′ at 70°C.

Since one of the principal objects of this study has been to gain information that may be of assistance in subsequent interpretations of the photolytic behaviour of nucleic acids[6], our efforts here were devoted to a comparison of the behaviour of the various analogues of cytosine, and particularly the nucleosides and nucleotides. A few additional points are, nonetheless, worth noting. Insofar as the reaction order is concerned we have found this, with one or two exceptions, to be unity as was previously reported by SINSHEIMER[2] for uridine. WANG *et al.*[5] present the photolysis of 1,3-dimethyluracil as a zero order reaction, but this is obviously due to the fact that they irradiated high concentrations such that 100% of the incident energy was absorbed during most of the course of the reaction. There are, however, one or two instances where we have found the reaction not to be strictly first order, the net result being a variation in quantum yields during the course of photolysis; such is the case for pyranosylcytosines at pH 13 and 14 (see Table I).

Although we have made no detailed study of the effect of concentration on the photolytic reaction (an increase of 5× in concentration does not affect any of the above reported results) we have found, during attempts to isolate the photolysis product of 2-methoxycytosine by irradiation of very high concentrations, that the nature of the reaction may be profoundly modified under these conditions. In addition, in buffered media the buffer itself apparently may have some effect not only on the photolytic reaction but on the subsequent reversibility as well. A case in point is cytosine (see above). A more striking example is that of galactopyranosylcytosine in 1 *M* Thorell buffer pH 11, in which the quantum yield for photolysis is lower than in 10^{-3} *N* NaOH, while the reverse reaction is considerably faster.

DISCUSSION

On the basis of a study of the rates of the forward (photolysis) and backward (dark) reactions in ordinary and heavy water for cytosine, cytidine and 1-methyluracil, it

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was concluded that, as for 1,3-dimethyluracil[4,5], the photochemical reaction in the case of cytosine and its nucleosides and nucleotides involves also the addition of a water molecule across the 5:6 double bond with the OH group in position 6[6]. Table II presents some of the results and, for comparison, SINSHEIMER's [2] values of Φ for uracil and uridine. It should be noted, in particular, that the photolysis of thymine, which is *not* reversible, proceeds with the lowest quantum yield in ordinary water; in addition, in contrast to those compounds which exhibit reversible photolysis, its quantum yield in heavy water increases instead of decreasing.

TABLE II

QUANTUM YIELDS FOR PHOTOLYSIS OF SEVERAL PYRIMIDINE DERIVATIVES

Compound	Φ (mole/einstein $\times 10^3$)	
	in D_2O	in H_2O
Uracil	6.0	5.2**
Uridine	22	22**
1-Methyluracil	5.7	12.5
Cytidine	4.2	9.0
Cytosine	0.7	1.7
Thymine	0.9*	0.4*

* Reaction not reversible in the dark.
** Values given by SINSHEIMER[2].

The fact that substitution of a methyl group on the number 1 nitrogen of cytosine does not markedly alter the quantum yield (in the case of uracil this is not so, Φ for 1-methyluracil being twice that for uracil) suggests that the replacement of the methyl group by a sugar group, which does not fundamentally alter the character of the $N_{(1)}$–$C_{(1')}$ bond, will also not substantially affect the course of the photochemical reaction. Hence, if the reaction mechanism involves the addition of a water molecule across the 5:6-bond of the pyrimidine ring, the higher quantum yields for nucleosides and nucleotides (Table I) indicate that, in the excited state, these molecules more readily take up water as a result of some strong intramolecular forces, resulting in such a distribution of electron density in the cytosine ring that the electron density on carbon 6 is lower than in the molecules of cytosine and 1-methylcytosine in their excited states.

The existence of strong intramolecular forces between the pyrimidine and carbohydrate rings in cytosine nucelosides may be inferred from an examination of the spectra of these compounds in neutral solution, as compared to that of 1-methylcytosine[8]; the principal differences involve appreciable variations in the pK values of the amino groups, and the tendency towards the formation in the nucleosides of an additional maximum in the region 2300–2400 which is most clearly resolved in the case of pyranosylcytosines[8] (see also Fig. 5, spectrum for non-irradiated galactopyranosylcytosine). In acid solution, on the other hand, the spectra of these substances are strikingly similar to each other and to that of 1-methylcytosine, suggesting a uniform structure for all of them; it is therefore hardly accidental that the quantum yields for all these compounds in acid solution are likewise almost identical (Table I). Another striking fact is that in the pH region 6–10.5, where the quantum yields for group (b) compounds is almost one order of magnitude greater than for group (a), irradiation results in the appearance of a new maximum at 2300–2360 A which in the case of

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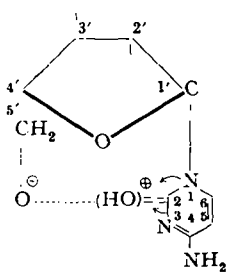
pyranosylcytosines clearly represents merely an increase in the height of the already existing one. It is also probably not accidental that pyranosylcytosines, which exhibit the largest degree of interaction as judged by the clearly resolved maximum at 2360 A of the normal compound, also exhibit the highest quantum yields for photolysis.

The comparable values of Φ for the enol form of cytosine (pH 14, Table I), methoxycytosine in neutral solution* and nucleosides and nucleotides in the pH range 6–11 (with the exception of desoxycytidylic acid), suggest a similar electron distribution density in the cytosine ring of all these compounds. While for the enol form of cytosine and for 2-methoxycytosine there is little doubt about this, it is of course possible that for the entire group of compounds this applies to them only in the excited state (see, however, below). When to this is added the observation that Φ for group (b) compounds decreases appreciably upon dissociation of the carbohydrate hydroxyls, it appears not unreasonable to expect that the factor responsible for increasing the rate of uptake of a water molecule is some kind of strong interaction between the carbonyl group of cytosine and one of the carbohydrate hydroxyls. Such interaction leads to a similar distribution of electron density in the pyrimidine ring as the substitution of a 2-methoxy group for the carbonyl group in cytosine, or the enolization of cytosine. The form of this interaction is probably that of a strong hydrogen bond between the cytosine carbonyl and a carbohydrate hydroxyl $C_{(2)} = O \ldots (HO)C'$, and most probably the 5′ hydroxyl. Supporting such a suggestion are the findings of CLARK, TODD AND ZUSSMAN[11] and ANDERSEN, HAYES, MICHELSON AND TODD[12] on O^2:5′-cyclocytidine and 2′-desoxy-O^2:5′-cyclocytidine, as well as the relatively low Φ for desoxycytidylic acid in which the 5′ hydroxyl is esterified. It is, however, not possible to exclude participation of the 2′ and 3′ hydroxyls in view of the difference in photochemical behaviour of desoxycytidylic acid which exhibits a lower Φ than the other compounds of group (b), but still higher than that of cytosine. In all likelihood, involvement of these groups is of lesser importance, although cyclonucleosides of uracil and thymine have been shown to involve the 2′ and 3′ hydroxyls as well [13,14,15]. A study of the various isomeric and cyclic phosphates of cytidine might be expected to clarify this question and such a study is being undertaken.

The observations of TODD *et al.*[11,12] eliminate the possibility of the photochemical formation of a covalent bond such as in cyclocytidine, since such a compound is unstable, its weakest point being the glycosidic linkage $N_{(1)}$—$C_{(1')}$. In addition such a photoproduct would differ from cyclocytidine in that the 5:6 double bond would be reduced, thus resulting in an even weaker glycosidic linkage in accordance with the normal behaviour of 5,6-dihydronucleosides.



The concept of a strong hydrogen-like bond makes it possible to explain the more ready uptake of water by the 5:6 double bond, since the oxygen of the carbonyl group of cytosine becomes positively charged, as shown in the accompanying diagram. The increase in the inductive effect of the carbonyl group results in

* For 2-methoxycytosine Φ is $10 \cdot 10^{-3}$ at pH 7.2. This compound is one of several 2- and 4-methoxy derivatives of cytosine and uracil which give relatively stable photoproducts at neutral pH, apparently without rupture of the pyrimidine ring. The behaviour of 2-methoxycytosine in ordinary and heavy water is similar to that of cytosine both for the forward and backward reactions (work in progress).

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a diminution of the electron density on the number 6 carbon, facilitating in this way the nucleophilic attachment of a water molecule at 5:6.

The above explains also the behaviour of nucleosides and nucleotides in alkaline medium, since dissociation of the carbohydrate hydroxyls results in a destruction of the hydrogen bonds, and hence a decrease in Φ. On the other hand the lack of reversibility in very alkaline medium (pH 13–14) is due to the instability of 5:6-dihydro derivatives under these conditions, which cause an opening of the pyrimidine ring[16].

Such a type of hydrogen bonding in cytosine nucleosides is in disagreement with that proposed by FURBERG[17], who suggests hydrogen bonding between $C_{(6)}$ and the 5′ hydroxyl, a rather unlikely hypothesis in view of the nature of the $C_{(6)}$—H bond (and lack of a labile hydrogen). It must however be borne in mind that FURBERG's conclusions were based on a study of crystals and are not necessarily applicable in solution. The findings of TODD *et al.* [11-15], referred to above, indicate that in cyclic pyrimidine nucleosides the (covalent) bond between the pyrimidine and carbohydrate rings involves the number 2 carbonyl group in the pyrimidine ring.

Returning to the absorption spectrum of the photoproduct it appears, in view of the above, that the differences between the two groups of compounds has its source in the hydrogen bonding of the carbonyl oxygen and the sugar 5′ hydroxyl. The character of such a hydrogen bond is partially that of an interaction of the type of a π-donor-acceptor hydroxyl hydrogen with a carbonyl group, at the expense of the π-electrons of the latter. An interaction of such a type should result in an extension of the system of π-electrons and a displacement of the absorption maximum towards the red, in relation to 5-hydro-6-hydroxy derivatives of cytosine and 1-methylcytosine. The latter two compounds do not exhibit a maximum in the range 2150-3000A, like the keto forms of dihydro derivatives of thymine and uracil; the enol forms of the latter two derivatives do exhibit a maximum in the neighbourhood of 2300 A[16]. It is therefore probable that the maximum of the photoproduct at 2360 A involves a similar electron transition. On this basis the maximum (or point of inflexion) at 2300–2370 in the spectra of cytosine nucleosides and nucleotides would be the result of a similar electron transition with, however, a lower degree of probability as indicated by the fact that the molar extinction is only about one-half that of the photoproduct.

The implications of the above results, as well as those of SINSHEIMER[2] on uridylic acids, for the photochemistry of nucleic acids are of undoubted interest. Table II shows how low Φ is for thymine as compared to uracil and cytosine. Our observations on 5-methylcytosine indicate an equally low quantum yield for this compound, as well as a lack of reversibility. SINSHEIMER[2] reports that thymidylic acid is about 1/200 as sensitive to irradiation as uridylic acid; our own results indicate a quantum yield 1/20 that for uridylic acid, but this is of no major importance. The fact is that thymidylic acid is much more resistant to radiation than uridylic and cytidylic acids. It is reasonably safe to conclude that 5-methyl cytidylic and desoxycytidylic acids are equally resistant to irradiation. In addition, numerous observers have noted that purines, as well as their nucleotides, are considerably more resistant to irradiation than pyrimidines[18,19,20]. It therefore follows that those components of nucleic acids which are most sensitive to radiation, *viz.* uracil and cytosine nucleotides, are at the same time those which exhibit the phenomenon of reversibility. It is therefore by no means unreasonable to expect that some degree of reversibility may be exhibited by nucleic acids[6]. Despite the low quantum yield for desoxycytidylic acid (Table I) the behaviour of desoxyribo-

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nucleic acid should resemble that for ribonucleic acid. It must of course be borne in mind, from the results presented above, that secondary linkages in the nucleic acid chains, involving the pyrimidine rings, may considerably influence the degree of reversibility for individual nucleotides. At any rate the biological implications of this phenomenon certainly warrant further studies along these lines.

We should like to thank Mgr. P. BLICHARSKI for his assistance with syntheses, and Prof. J. HELLER for his kind interest in this and related studies.

SUMMARY

1. Ultraviolet irradiation of cytosine, 1-methylcytosine and nucleosides and nucleotides of cytosine under suitable conditions leads to the formation of unstable products which may be made to revert to the original substance.

2. The photochemical reaction probably involves the addition of a water molecule to the 5:6 double bond of the pyrimidine ring, as for uracil derivatives.

3. Quantum yields are presented for the above compounds over a wide range of pH values. A comparison of quantum yields with the absorption spectra of the various compounds and their photoproducts indicates that hydrogen bonding between the pyrimidine and carbohydrate rings plays an important role in the reaction.

4. A comparison of the behaviour of 1-methylcytosine and cytosine nucleosides (and nucleotides) suggests that hydrogen bonding involves the pyrimidine carbonyl and the carbohydrate hydroxyls, and principally the 5′ hydroxyl.

5. The implication of the results, as regards the photochemistry of nucleic acids, is pointed out and discussed.

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