THE WAVELENGTH DEPENDENCE OF SOME EFFECTS OF ULTRAVIOLET RADIATION ON IN VITRO DNA OF PHAGE α

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ABSTRACT The wavelength dependence of some of the effects of ultraviolet radiation on the physicochemical properties of DNA of phage α irradiated in vitro are discussed. An analytical ultracentrifuge and a spectrophotometer were used to study (*a*) the breaking of individual polynucleotide strands; (*b*) the local denaturation; (*c*) the presence of a fraction of molecules resistant to denaturation; and (*d*) the increase in the buoyant density of irradiated DNA. All the curves show a slight variation of the radiation efficiency in the range 2600–2800 A, and a well defined peak at λ = 2880 A.

INTRODUCTION

Ultraviolet radiation has been employed in recent years to study the physicochemical properties of DNA and its constituents. Thymine dimers were the first main photoproducts of UV radiation to be described, and they have been found both in frozen solutions of thymine (Beukers, Ijlstra, and Berends, 1958; Wang, 1961) and in aqueous solutions of poly T (Deering, 1962) and DNA (Beukers and Berends, 1960, 1961). Recently good evidence has been obtained for the same irradiation photoproduct in DNA irradiated in vivo (Wacker, Dellweg, and Weinblum, 1960; Wulff and Rupert, 1962; Setlow, Swenson, and Carrier, 1963; Setlow and Carrier, 1964; Boyce and Howard Flanders, 1964). For low UV doses, it has been concluded that about 50% of the UV damage to bacteriophage is due to thymine dimer formation (Sauerbier and Haug, 1964; Sauerbier, 1964).

Recently cytosine photoproducts have also been found, although not all of them have yet been isolated. The final product of cytosine irradiation seems to be a stable uracil-thymine dimer from irradiated DNA (Setlow, Carrier, and Bollum, 1965).

In addition, UV radiation produces partial local denaturations and breakages in the DNA molecule and cross-links between strands, but these effects have been obtained only with high radiation doses.

So far, the correlation of these different phenomena has not been studied in detail, so we investigated the wavelength dependence of the UV damage done to DNA

molecules in vitro in an attempt to separate the different reactions and to see if they were correlated in any way.

MATERIALS AND METHODS

Deoxyribonucleic Acid. DNA (mol wt 35×10^6) was extracted from phage α (Aurisicchio et al., 1962) (active on *Bacillus megatherium*) by the method described by Mandell and Hershey (1960). This material is particularly suitable for studying UV effects on the single strands of the molecule, since, after denaturation, its two strands have different densities in a CsCl gradient, owing to their different base composition (Tocchini-Valentini et al. 1963).

Ultraviolet Irradiation. DNA solutions were irradiated using a Hilger quartz prism monochromator. Three ml of stirred solution (DNA_α 20 μg/ml in 0.15 N NaCl) were irradiated at room temperature in a quartz cuvette of 1 cm light path.

The samples were irradiated at different wavelengths using the same absorbed dose ($D_{abs} = 3.2 \times 10^{14}$ quanta/mm^3 for the samples destined for the analytical ultracentrifuge and $D_{abs} = 6.4 \times 10^{14}$ quanta/mm^3 for the ones used in the spectrophotometer analysis) and the same $\Delta\lambda = \pm 10$ A. The irradiation times were in the range 10 min ($\lambda = 2600$ A) to about 120 min ($\lambda = 3000$ A).

The absorbed dose was evaluated from the expression

$$D_{abs} = D_{inc}(1 - T),$$

where the incident dose D_{inc} was measured by the thermopile galvanometer method, and the transmission coefficient T was determined spectrophotometrically.

One sample, in a Petri dish, was irradiated with a very high dose ($D_{abs} = 10^{16}$ quanta/mm^3) at distance of 15 cm from a germicidal lamp having maximum emission at 2570 A.

Alkaline Denaturation. Prior to CsCl density gradient centrifuging, the irradiated DNA samples were denatured, bringing their pH to 12.4 by adding 5 N NaOH. After 5 min the pH was adjusted to 7.0 by adding HCl 2.5 N (Ageno, Dove, and Frontali, 1966).

Thermal Denaturation Thermal denaturation was recorded by means of the absorbance-temperature curve at 2600 A, which was determined with a Beckman spectrophotometer (model DU) equipped with a thermospacer.

Sedimentation. Sedimentation coefficients of the DNA samples were determined at 42,020 *g* in a Spinco model E ultracentrifuge using ultraviolet absorption ogtics.

CsCl Density Gradient Centrifuging. After alkaline denaturation, solid CsCl was added to the DNA solution until a refractive index of $n = 1.4020$ was obtained, and then the solution was centrifuged at 44,770 *g* for about 24 hr. The DNA of *B. megatherium* was used as a reference ($\rho_0 = 1.695$ g/cm^3). The resulting photographs were surveyed with a microphotometer and its tracings were analyzed as a set of gaussian curves, using the Montecarlo method adapted for the 7040 IBM computer (Cortellessa and Farchi, 1965).

The errors of the parameters obtained by this analysis were calculated on the computer from the difference between the theoretical gaussian curves and the experimental data.

The densities of the banded DNA were determined from the values corresponding to the maxima of the gaussian curves, using Sueoka's relationship (Sueoka, 1959). The average number of breaks per molecule was derived from the gaussian half width σ, using the theory of Montroll and Simha (1940), which is valid only if the breaks are randomly distributed both among the molecules and along the single molecule.

In this case we have the relationship

$$p\left(\frac{\sigma_0}{\sigma}\right)^2 = 1 + 2\frac{e^{-\alpha p} + \alpha p - 1}{\alpha^2 p},$$

where α is the average degree of depolymerization, and p is the number of bonds that may be broken.

RESULTS

Lowering of the Sedimentation Coefficient

We examined the sedimentation coefficients of the DNA irradiated at various wavelengths in order to make sure that the doses of UV radiation did not break the double strands of the DNA. The results given in Table I show the inefficiency of the doses.

By increasing the doses it was possible to show appreciable changes in the sedimentation coefficients (see Table II).

TABLE I

SEDIMENTATION COEFFICIENTS OF PHAGE α DNA AFTER IRRADIATION AT DIFFERENT WAVELENGTHS

($D_{abs} = 6.4 \times 10^{14}$ quanta/mm^3)

$\lambda \pm \Delta\lambda$	$s_{20,w}$
A	*Svedberg*
Control	32 ± 1
2600 ± 10	32 ± 1
2700 ± 10	32 ± 1
2800 ± 10	32 ± 1
2850 ± 10	30 ± 1
2900 ± 10	33 ± 1
2950 ± 10	30 ± 1
3000 ± 10	32 ± 1

TABLE II

SEDIMENTATION COEFFICIENTS OF PHAGE α DNA AFTER IRRADIATION AT λ = (2600 ± 10) A WITH DIFFERENT DOSES

D_{abs}	$s_{20,w}$
Quanta/mm^3	*Svedberg*
Control	32 ± 1
6.0×10^{14}	32 ± 1
1.2×10^{15}	32 ± 1
3.0×10^{15}	25 ± 1
5.0×10^{15}	21 ± 2
8.0×10^{15}	10 ± 2

Samples of DNA were irradiated with $D_{abs} = 3.2 \times 10^{14}$ quanta/mm³ and, after alkaline denaturation, were examined in a CsCl gradient and analyzed as a sum of gaussian distributions, as described above.

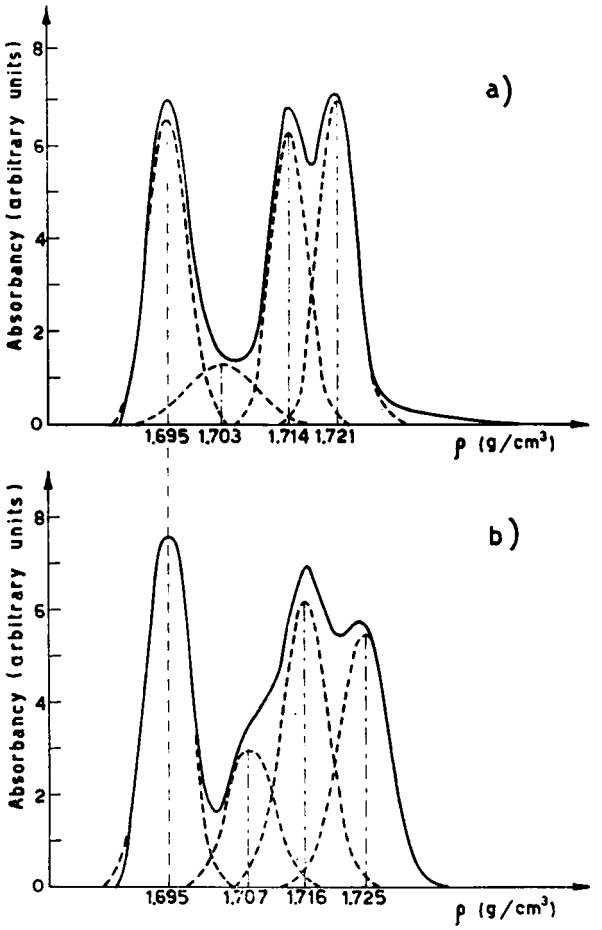

FIGURE 1 Effect of UV radiation on the CsCl buoyant density of phage α DNA: (a) control ($\rho = 1.695$, *Bacillus megatherium* DNA employed as the density marker; $\rho = 1.703$, residual nondenatured DNA; $\rho = 1.714$, light strand; $\rho = 1.721$, heavy strand); (b) irradiated at $\lambda = (2820 \pm 10)$ A, $D_{abs} = 3.2 \times 10^{14}$ quanta/mm³. Solid curve: microphotometer trace; dashed curve: analysis as a set of gaussian curves.

Fig. 1 b displays an example of the results obtained for a sample of DNA irradiated at $\lambda = (2820 \pm 10)$ A, and Fig. 1 a gives the results from the unirradiated control.

In the irradiated sample a well defined band can be seen at $\rho = 1.707$, corresponding to about 25% of the total DNA, which can be ascribed to a fraction of nondenatured DNA. This band shows a density increment with respect to the control.

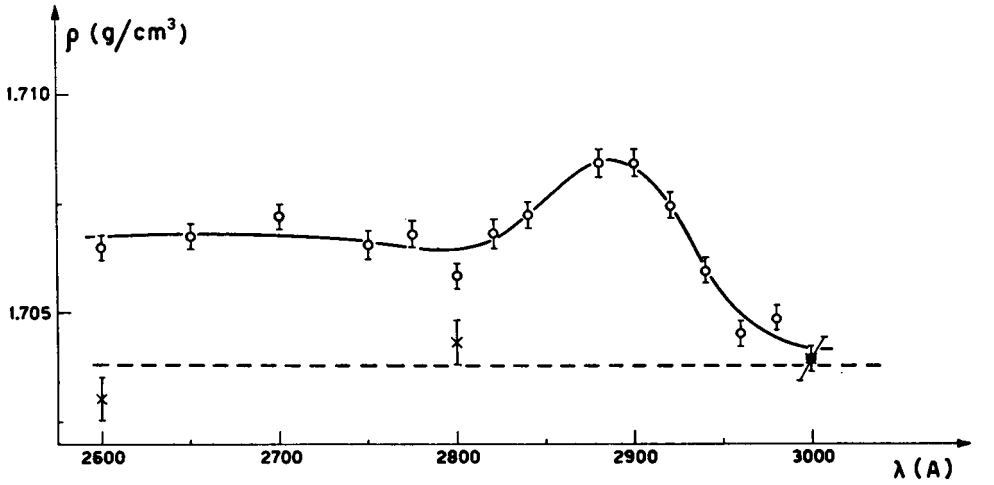

FIGURE 2 CsCl buoyant density of the nondenatured fraction of phage α DNA as a function of wavelength. Dashed curve: CsCl buoyant density of the native nonirradiated DNA.

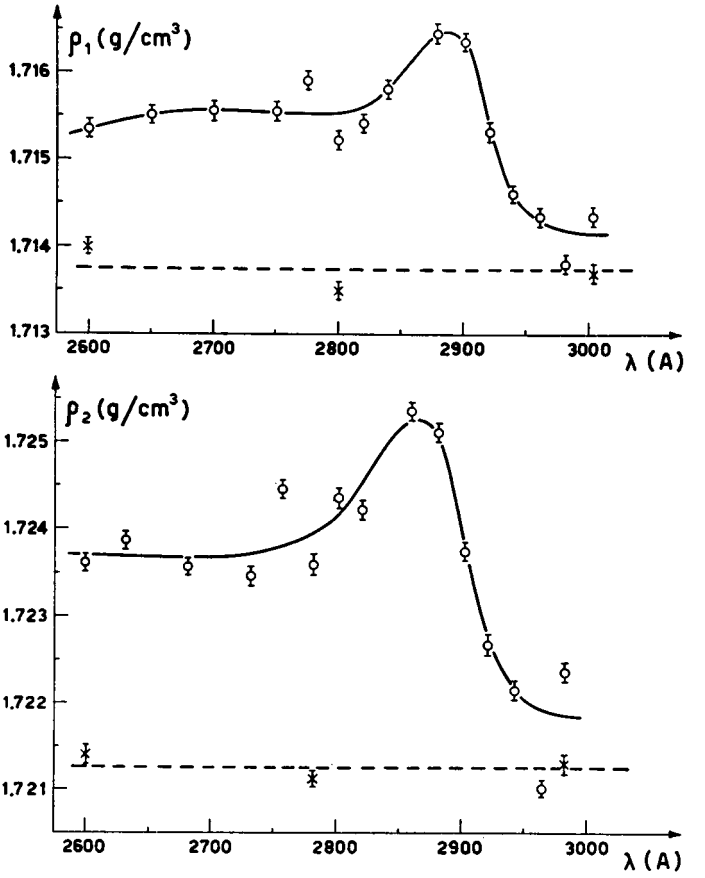

FIGURE 3 CsCl buoyant density of the light strand (ρ_1) and heavy strand (ρ_2) of phage α DNA as a function of wavelength. Dashed curve: densities of the nonirradiated strands.

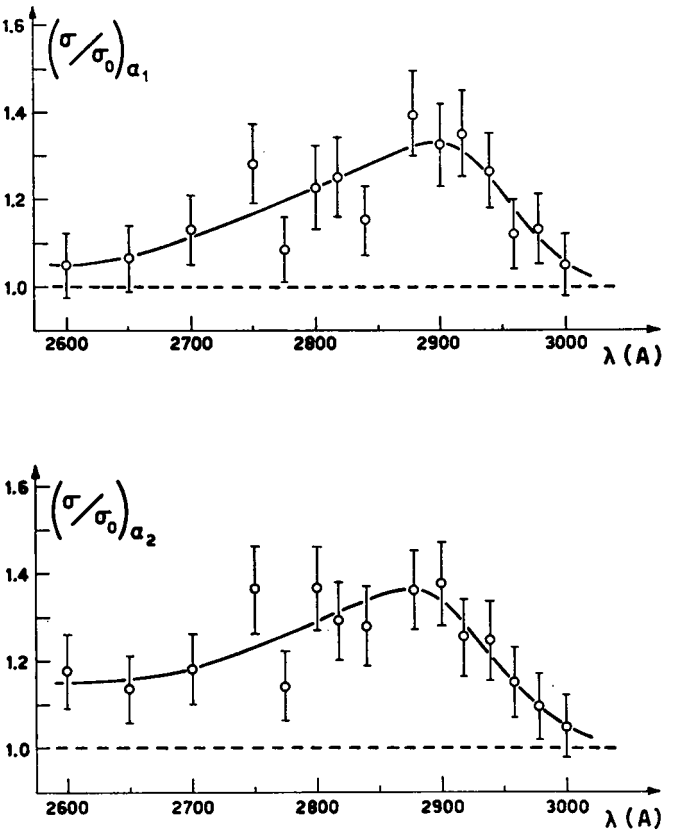

FIGURE 4 Relative broadening of the gaussian curves corresponding to the light ($(\sigma/\sigma_0)_{\alpha_1}$) and heavy ($(\sigma/\sigma_0)_{\alpha_2}$) strands of phage α DNA as a function of wavelength.

The two bands of the denatured DNA present a similar density increment, with a greater increment in the heavy component. In addition, the width of the two corresponding gaussian distributions is greater than that of the control ones, which means that the homogeneity of the component materials is poorer and that breaks in the individual strands of DNA have been produced. These studies have been repeated at 15 different wavelengths, and the densities of the nondenatured fraction and of the single strands of the irradiated DNA are given in Fig. 2 and Fig. 3. These curves show an approximately constant efficiency between 2600 and 2750 A, and a peak at $\lambda = 2880$ A; furthermore, the efficiency is greater with the denser strand.

For $\lambda \simeq 3000$ A the efficiency seems to be zero, but at this wavelength the measurement of the absorption coefficient of the DNA preparation has such large errors that the data can be regarded only as qualitative.

From the same analysis of the gaussian curves one can obtain the relative broadening of the two components of the DNA (see Fig. 4). For the more efficient wavelength (λ = 2880 A), the average number of breaks for a single strand, calculated as described under Materials and Methods, is 2. This corresponds to a quantum efficiency

$$\phi_{\lambda=2880\,A} = 4 \times 10^{-6} \text{ breaks per absorbed quantum.}$$

Optical Density–Temperature Curves

Samples of DNA irradiated with a $D_{abs} = 6.4 \times 10^{14}$ quanta/mm^3 were examined spectrophotometrically, as previously described. In Fig. 5 the optical density–

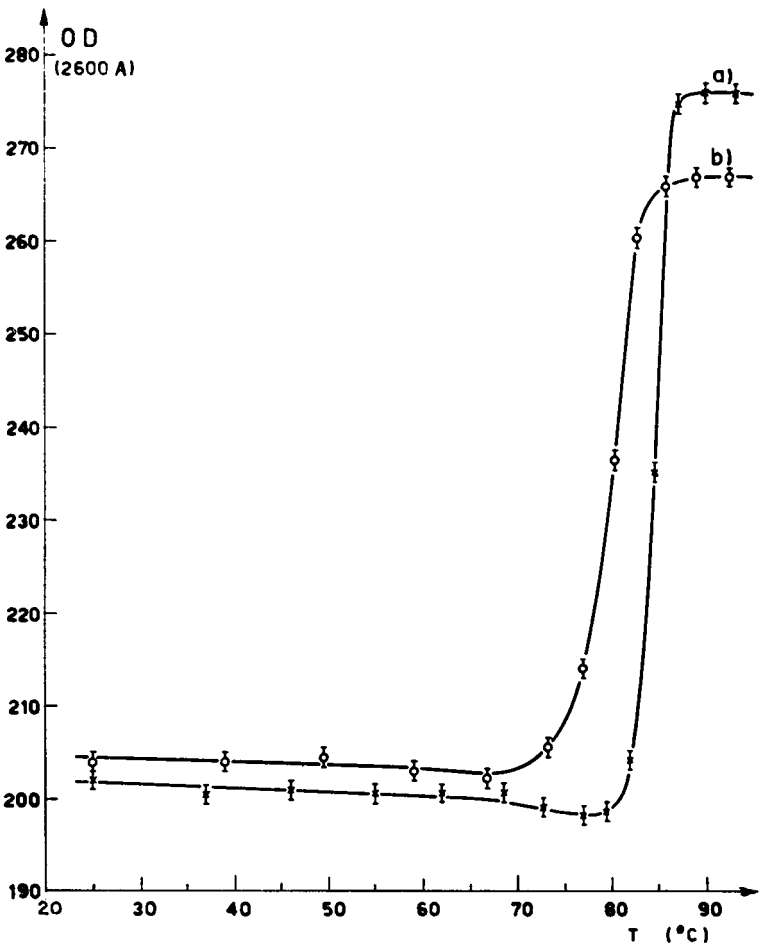

FIGURE 5 Effect of UV radiation on the optical density–temperature curves of phage α DNA: (a) native; (b) irradiated at λ = (2820 $\pm$ 10) A, D_{abs} = 6.4 $\times$ 10^{14} quanta/mm^3.

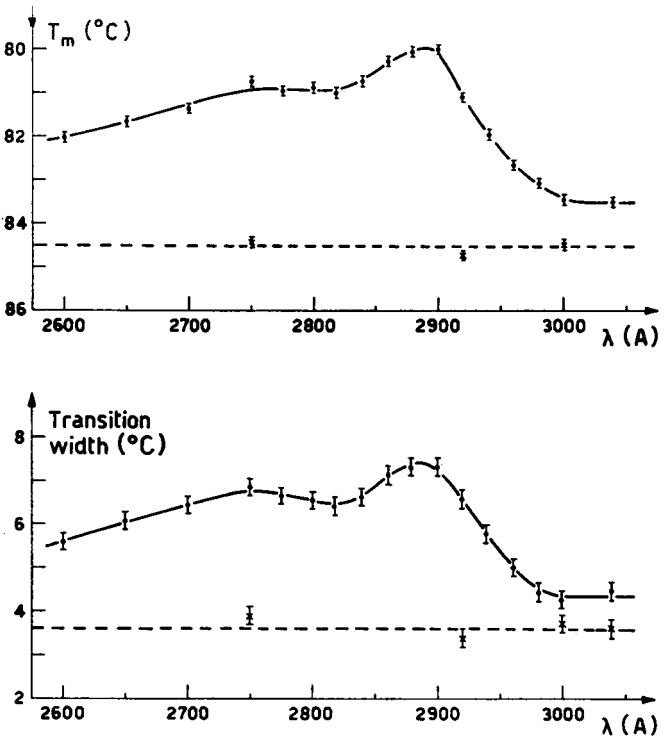

FIGURE 6 Melting temperature and transition width (ΔT between 10% and 90% of the optical density increase) of the thermal transition as a function of wavelength. Dashed curve: values for nonirradiated samples.

temperature curves of a sample, irradiated at $\lambda = (2820 \pm 10)$ A, and of a control are shown. It can be seen that the hyperchromic effect is slightly diminished, whereas the melting temperature, T_m, and the transition width (defined as the temperature interval ΔT between 10% and 90% of the optical density increase) change from 84.5°C and 3.6°C to 80.0°C and 7.2°C, respectively.

Similar curves were determined for all irradiated samples. In Fig. 6 the melting temperatures and the transition widths are reported as a function of wavelength. The shape of the two curves is the same, and very similar to that of the curves in Figs. 2 and 3. There is a maximum at $\lambda = 2880$ A and a region of slightly varying efficiency between 2600 and 2800 A.

We also irradiated a sample with very high doses ($D_{abs} = 10^{16}$ quanta/mm^3) using a germicidal lamp, and the modifications of the optical density–temperature curves are shown in Fig. 7. It is apparent that the transition is now gradual and no longer sharp as before.

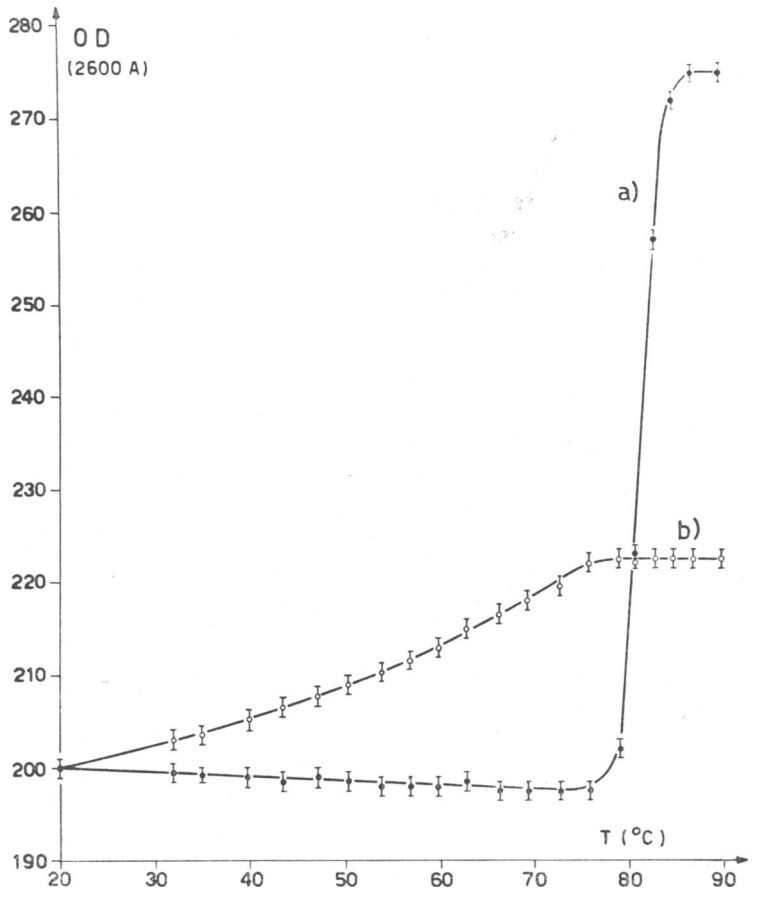

FIGURE 7 Optical density–temperature curves of phage α DNA: (a) native; (b) irradiated with a germicidal lamp, $D_{abs} = 10^{16}$ quanta/mm^2.

DISCUSSION

Within the limits of the experimental procedure of treating irradiated DNA with alkali, and over the range of our doses, the above data seem to show that radiation causes some breaks in the individual strands of native DNA. From the behavior of the ratio σ/σ_0 (Fig. 4) we have seen that at $\lambda = 2880$ A the quantum yield for the single-strand breaks is $\phi_b = 4 \times 10^{-6}$ breaks per quantum absorbed: this wavelength corresponds to an energy of about 4.3 ev.

This quantum yield may be compared with that of $\phi \simeq 10^{-6} - 10^{-5}$ found by Greendstein and Jenrette (1941) and by Hollaender (Hollaender, Greendstein, and Jenrette, 1941) for the decrease in viscosity of irradiated DNA. More recent studies (Alexander and Moroson, 1960) have shown that the initial effect of UV radiation

results in a decrease in viscosity with no change in molecular weight; these data strongly support the evidence for single-strand breaks.

If this is the case, the double-strand breaks that are observed (Table II) may be the result of individual single-strand breaks which, at high doses, appear as a double ones because they are close together and because the melting temperature has been decreased.

We have seen that UV causes a slight diminution of the hyperchromic effect and a lowering of the T_m value. These effects, already noted by others (Marmur et al., 1961), may be due to some broken hydrogen bonds which were near to the breaks mentioned above. In this case, if we suppose that the melting energy depends on the number of consecutive hydrogen bonds, we obtain a curve very like that in Fig. 5.

Other effects of UV radiation are the presence of a nondenatured fraction of DNA (Fig. 1) and the increase in the mean density both of this fraction and of the two strands of the DNA, with a greater effect on the denser one. These shifts in density are probably due to thymine dimers, the formation of which has a maximum at $\lambda \simeq 2800$ A (Wulff, 1963); this hypothesis is supported by the work of Marmur et al. (1961). According to this hypothesis the different sensitivities of the two strands would be due to their different base composition—32.1% thymine in the heavy strand as compared with 24.5% in the light one—which implies a relative probability of dimer formation of 10% and 6%, respectively. The shift in density could also be due to the formation of cytosine dimers, but then it would be difficult to explain the difference in sensitivity of the two strands of the phage α DNA.

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