VARIATION IN THE PHOTOCHEMICAL REACTIVITY OF THYMINE IN THE DNA OF *B. SUBTILIS* SPORES, VEGETATIVE CELLS AND SPORES GERMINATED IN CHLORAMPHENICOL*†

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Abstract—The chief photoproduct of thymine produced in u.v. irradiated (2537Å) vegetative cells of *B. subtilis* is the cyclobutane-type dimer while in spores very little of this dimer is produced (maximum yield 2·6 per cent of thymine) but a new photoproduct is produced in high yield (maximum of 28·4 per cent of thymine). This difference in photochemical response appears to be due, at least in part, to a difference in uydration of the DNA. The photochemistry of thymine in isolated DNA irradiated in solution is similar to that of DNA in irradiated vegetative cells, but differs markedly from that of isolated DNA irradiated dry. The yield of cyclobutane-type thymine dimer is much reduced in isolated DNA irradiated dry but a new photoproduct of thymine is produced which is chromatographically similar to the spore photoproduct. The yield of this photoproduct, however, is never as great as that obtained in irradiated spores.

The photochemistry of the DNA thymine of spores germinated in the presence of chloramphenicol is very similar to that of normal vegetative cells. Except for hydration, the physical state of the DNA is probably not otherwise altered by germination in the presence of chloramphenicol since DNA replication is prevented by the presence of chloramphenicol. These results are also consistent with the hypothesis that the unique photochemistry of spores is due, at least in part, to the hydration state of the DNA.

The acid stability of the spore photoproduct is indicated by the fact that it is isolated from irradiated spores after hydrolysis in trifluoroacetic acid at 155°C for 60 min. It still contains the methyl group of thymine as judged by the fact that for a given dose of u.v. the same yield of photoproduct was obtained whether the spores were labeled with thymine-2-C-14 or -methyl-C-14. This photoproduct is stable to reirradiation (2537Å) in solution under conditions where thymine dimers of the cyclobutane-type are completely converted back to monomeric thymine. On a column of molecular sieve material (Sephadex-G10), the spore photoproduct elutes in a region intermediate between the cyclobutane-type thymine dimers and monomeric thymine. Of the numerous compounds tested by paper chromatography, the spore photoproduct is most similar (but not identical) in several solvents to 5-hydroxyuracil and 5-hydroxymethyluracil. Our data do not allow us to decide if the product is a monomer or a dimer.

Although the photochemistry of thymine in the DNA of spores differs markedly from that for vegetative cells, several lines of evidence make it seem doubtful that the enhanced resistance of spores to u.v. relative to that of vegetative cells can be explained solely on the basis of this difference in the photochemistry of DNA thymine.

INTRODUCTION

THE PHOTOCHEMICAL sensitivity of DNA is, in part, a function of its physical state. The rate of formation of thymine dimers in heat-denatured DNA is about twice that for native

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DNA.[1,2] The yield of thymine dimers is grealy depressed if DNA is irradiated dry.[3]*
Spores are less sensitive to u.v. irradiation than are vegetative cells.[4] The DNA of spores
is thought to be in a more arid state than in vegetative cells.[5] Recent reports have also
indicated that the photochemistry of DNA thymine is markedly different in spores and in
vegetative cells of *B. megaterium*.[6–8]

The DNA of spores of *Bacillus subtilis* that have been germinated in the presence of
chloramphenicol (and therefore the initiation of DNA synthesis has been prevented)
does not function as a primer for DNA polymerase action until treated with deoxyribonu-
clease for a short time.[9] The DNA of polyoma virus is present in a "super-coiled" state
which does not assume the double stranded circular shape until one single strand break
has been introduced into the molecule by pancreatic deoxyribonuclease or chemical
reducing agents.[10] If the DNA of spores were in a "super-coiled" state, this might
explain why it does not function properly as a primer for DNA polymerase activity until
a change in its physical state is brought about by preliminary treatment with deoxyribonu-
clease.

This hypothesized change in the physical state of the spore DNA brought about by
treatment with deoxyribonuclease might be followed as a change in the photochemical
reactivity of the DNA and might be a more sensitive criterion than physical measurements
upon isolated DNA since the DNA need not be isolated prior to the exposure to u.v.
We therefore compared the photochemical response of the DNA thymine of *B. subtilis*
vegetative cells, spores, and spores germinated in the presence of chloramphenicol with
and without subsequent treatment with deoxyribonuclease in an effort to determine at
what stage in the germination cycle the physical state of the DNA of spores is altered in
the hope that it may help to explain (1) why the DNA of spores germinated in the presence
of chloramphenicol does not function as a primer for DNA polymerase action until treated
with deoxyribonuclease for a short time, and (2) determine if there is a photochemical
explanation for the enhanced u.v. resistance of spores.

METHODS

A mutant of *B. subtilis* 168 that requires thymine, indole and leucine (MY2Y1U2) was
used. For preparation of thymine-C-14 labeled spores, cells were grown in synthetic
medium[11] supplemented with thymine-2-C-14 (25·2 mc/mM, Calbiochem) carrier-free
at 0·4 μc/ml, and L-leucine and L-tryptophan at 50 μg/ml. A 250 ml culture was shaken
in a 2-l. flask at 37°C for 4 days. Sporulation was followed by microscopic examination.
The spores and residual cells were harvested by centrifugation. The cells were lysed with
1 mg/ml lysozyme for 60 min at 37°C followed by treatment with 1% sodium lauryl sulfate
at 37°C for 30 min. The spores were harvested and washed 5 times with distilled water.
The specific activity of the spores was 18,300 counts/min per unit of O.D. at 650 mμ.

The procedure for the germination of spores in the presence of chloramphenicol has
been described.[9] Under these conditions no incorporation of leucine-C-14 into protein
could be detected and no replication of DNA took place.

The techniques used for the ultraviolet irradiation (2537Å) of cells, their subsequent
hydrolysis in trifluoroacetic acid and chromatographic analysis have been described.[12]

Cells were grown in mineral medium[13] supplemented with 2 μg/ml of thymine-
2-C-14 (24·3 mc/mM; Calbiochem), 50 μg/ml of L-leucine and L-tryptophan and glucose

*See this paper.

at 0·5%. Late log phase cells were irradiated in growth medium for the times indicated, made to 5% trichloroacetic acid (TCA) washed several times in cold 5% TCA, then several times with ethanol/ether (3/1) at room temperature and once at 60°C for 10 min., and then hydrolyzed.

Spores were irradiated in water and then processed as described for the cells.

To isolate DNA from vegetative cells, they were suspended in 3·5 ml of 0·05 M tris-buffer at pH 8·1 and an equal volume of lysozyme–EDTA solution (1 ml of 2 mg/ml lysozyme, Worthington, plus 4 ml of 1 mg/ml EDTA) and incubated for 5 min. at 37°C; 7 ml of 4% sodium lauryl sulfate was then added, and the clear solution stirred for 60 min. at room temperature. The procedure outlined by Smith[14] was followed subsequently. The isolated mixture of DNA and RNA was treated with ribonuclease (Worthington) at 0·36 mg/ml for 60 min. at 37°C, an equal volume of 4% sodium lauryl sulfate was added and the solution stirred at room temperature for 60 min. This was followed by the KCl treatment and alcohol precipitation steps in the DNA isolation procedure.[14] The DNA was finally dissolved in water (4 O.D.^{260} units/ml) and had 1·14 × 10^5 counts/min per O.D.$_{260}$ unit. This solution was diluted approximately 1 : 3 for irradiation in solution. After irradiation, the DNA was precipitated with alcohol and acid hydrolyzed. DNA samples (0·25 ml of stock solution) were also evaporated to dryness in a vacuum desiccator and irradiated dry. These samples were taken up directly in trifluoroacetic acid for hydrolysis. A second preparation of DNA (Sample No. 2 in Table 3) was used at a concentration of 0·8 O.D.$_{260}$ units/ml and had 1·83 × 10^5 counts/min per O.D.$_{260}$ unit (0·5 ml aliquots were dried for irradiation).

EXPERIMENTAL RESULTS

(1) *Photochemistry of thymine-C-14 labelled spores and vegetative cells*

About 45 ml of late log phase cells were irradiated with u.v. (2537Å) in growth medium for 90 min (4·7 × 10^4 ergs/mm^2) and 10 ml of spores (3 × 10^7/ml) were irradiated in water for 60 min (3·1 × 10^4 ergs/mm^2) and processed as described under Methods.

The photochemistry of thymine in the DNA of spores is markedly different from that for vegetative cells of *Bacillus subtilis*. Almost the only photoproduct of thymine that was produced in the cells was the thymine dimer (Table 1). Very little thymine dimer was

TABLE 1. PER CENT PRODUCTION OF PHOTOPRODUCTS OF THYMINE-2-C-14 IN CELLS AND
SPORES OF *B. subtilis*

Photoproducts	Cells	Spores
Origin area	0·1%	0·0%
Thymine dimer (R_f 0·26)		
(cyclobutane-type)	7·2%	2·7%
R_f 0·35	0·0%	11·4%

Ultraviolet dose: Spores, 3·1 × 10^4 ergs/mm^2; Cells, 4·7 × 10^4 ergs/mm^2.
Chromatographic solvent: n-Butanol–acetic acid–water (80:12:30) (Reference No. 15).
See text for further details.

formed in the irradiated spores, however, another photoproduct of thymine was formed in large amount. This material had an R_f of 0·35 in the butanol–acetic acid–water (80:12:30) solvent[15] compared to the dimer at 0·26 and thymine at 0·60. Similar results have been observed for *Bacillus megaterium*.[6–8]

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(2) *Photochemistry of thymine-C-14 labeled spores and spores germinated in chloramphenicol*

Normal spores and spores germinated in the presence of 100 $\mu g/ml$ chloramphenicol were irradiated for 90 min ($4 \cdot 7 \times 10^4$ ergs/mm^2), hydrolyzed and chromatographed.

The results for the normal spores (Table 2) are essentially the same as found in the previous experiment (Table 1). Very little thymine dimer was produced but a lot of the

TABLE 2. PER CENT PRODUCTION OF PHOTOPRODUCTS OF THYMINE-2-C-14 IN SPORES
AND SPORES GERMINATED IN CHLORAMPHENICOL

Photoproducts	Spores	Spores plus chloramphenicol
Origin	0.5%	0.2%
Thymine dimer (R_f 0.26) (cyclobutane-type)	3.8%	6.2%
R_f 0.35	12.5%	1.2%

Ultraviolet dose: $4 \cdot 7 \times 10^4$ ergs/mm^2 (2537Å)
See text for further details.

photoproduct at R_f 0.35 was produced. The results for the spores germinated in the presence of chloramphenicol, however, were very similar to those for vegetative cells (Table 1), that is, mostly thymine dimer was formed (Table 2).

Our expectation had been that the photochemistry of spores germinated in chloramphenicol would be identical to that for normal spores and would only change after the subsequent treatment with deoxyribonuclease. As shown above, the germination process itself was sufficient to alter the photochemistry. Treatment of the germinated spores with deoxyribonuclease prior to u.v. irradiation had no additional effect on the photochemistry of DNA.

(3) *Irradiation of DNA in vitro*

The chief photoproduct of thymine that was produced when DNA was irradiated ($4 \cdot 7 \times 10^4$ ergs/mm^2) in solution was the thymine dimer (Table 3). When the DNA was

TABLE 3. THE PHOTOCHEMISTRY OF THYMINE IN DNA IRRADIATED IN SOLUTION OR DRY

Photoproducts	Solution (Sample No. 1)	Dry (Sample No. 1)	(Sample No. 2)	Dry in 0.15 M NaCl (Sample No. 2)
Origin area	0.4%	5.7%	0.3%	0.8%
Thymine dimer (R_f 0.26) (cyclobutane-type)	6.1%	1.4%	2.1%	3.8%
R_f 0.35	0.6%	1.4%	3.0%	2.9%

Ultraviolet dose: $4 \cdot 7 \times 10^4$ ergs/mm^2 (2537Å).
Sample numbers refer to two different samples of *B. subtilis* DNA (See section on Methods).

irradiated while dry, the amount of thymine dimer produced was greatly reduced (confirming the results of Riklis[3]) and there was an increase in the amount of the 0.35 material produced. When DNA Sample No. 1 was irradiated dry, the major photoproducts chromatographed at or near the origin. At the lowest dose of u.v. ($2 \cdot 3 \times 10^4$ ergs/mm^2) principally material at R_f 0.01 was formed but at the higher dose ($4 \cdot 7 \times 10^4$ ergs/mm^2)

the amount of this material decreased and the material at R_f 0·04 showed a corresponding increase suggesting that the two materials are closely related. When DNA Sample No. 2 was irradiated dry almost no origin material was formed and the yield of dimer and R_f 0·35 material was increased. The DNA solution was somewhat more concentrated in Sample No. 1 (4 O.D.$_{260}$ units/ml) as compared to Sample No. 2 (0·8 O.D.$_{260}$ units/ml) and this may explain the difference in the amount of origin material formed. The two samples may also have been irradiated at a different humidity since dry Sample No. 1 was irradiated simultaneously with a sample in solution and dry Sample No. 2 was irradiated by itself. Drying DNA Sample No. 2 in 0·15 M NaCl (as compared to water used above) had only a slight effect upon its photochemistry (Table 3) (in contrast to the results of Donnellan and Setlow[6]).

(4) *Attempts to identify the photoproduct of thymine produced in spores*

A. Comparisons by paper chromatography. The spore photoproduct (arising from thymine-2-C-14) was chromatographed in several solvents along with numerous marker materials in an attempt at identification (Table 5).

Chromatographically the spore photoproduct is quite similar to 5-hydroxyuracil (isobarbituric acid). It cannot be this compound, however, since the photoproduct is formed with the same yield in irradiated spores labeled with thymine-methyl-C-14 or with thymine-2-C-14 (Table 4).

TABLE 4. YIELD OF THYMINE PHOTOPRODUCTS IN SPORES LABELED WITH THYMINE-2-C-14
OR THYMINE-METHYL-C-14

Photoproducts	Thymine-2-C-14	Thymine-methyl-C-14
Origin area	0·4%	0·7%
Thymine dimer (R_f 0·26)		
(cyclobutane-type)	1·7%	2·4%
R_f 0·35	21·3%	21·0%

Ultraviolet dose: 4·7 × 10⁴ ergs/mm².
Spores irradiated in water at about 10⁷ spores/ml.

Note: These two samples of spores were from a different preparation than the one
used in the experiments cited in Tables 1 and 2. Although these spores were
prepared as described under Methods, their appearance was different from
the first preparation in that they were much less pigmented (brown) than the
first preparation. This difference in pigmentation may explain the greater
yield of photoproduct per unit dose of u.v., but it is apparent the qualitative
photochemistry is not significantly different from the first batch of spores
(Tables 1 and 2).

The spore photoproduct is chromatographically similar to (but not identical with) the Type III isomer of the thymine dimer (Table 5). In this isomer the thymine rings are *cis* relative to the cyclobutane ring but the methyl groups are *trans*.[18] The chief isomer of thymine produced in DNA (Type 1) appears to be *cis* in both considerations.[17,18] The major evidence that the spore photoproduct cannot be the Type III cyclobutane-type thymine dimer is that the Type III dimer is converted back to thymine on reirradiation in solution[19] whereas the spore photoproduct is not altered under these conditions (as evidence by rechromatography after reirradiation), and the two photoproducts can be clearly distinguished from each other by gel filtration (see Section 4.C.).

TABLE 5. R_f VALUES

Compound	Chromatographic solvent				
	A	B	C	D	E
Spore photoproduct	0·35	0·21	0·56	0·49	0·49
Uracil	0·48	0·34	—	0·57[a]	—
Dihydrouracil	0·47	0·28	—	—	—
Urea	0·53	0·28	—	—	—
				0·48[a]	
5-Hydroxymethyluracil	0·35	0·27	0·64	0·51	—
				0·54[a]	
5-Formyluracil[c]	0·44	0·37	0·59	0·56	—
5-Carboxyuracil	—	—	—	0·08[a]	—
5-Hydroxyuracil	0·36	0·25	0·56	0·47	—
				0·68[a]	
Thymine	0·60	0·55	0·78	0·72	0·59
Dihydrothymine	0·61	0·53	0·79	0·64	—
Uracil dimer	0·12	0·03	—	—	—
Thymine–uracil dimer	0·19	—	—	—	—
		0·12[b]			0·49[b]
Thymine dimer (Type I)[d]	0·26	0·14	—	—	0·49
(Type III)[d]	0·33[e]	0·17[b][e]	0·62[e]	0·40[c]	0·56[b][e]

Chromatographic Solvents:

   A.   n-Butanol–acetic acid–water (80/12/30).
   B.   n-Butanol–water (86/14).
   C.   Isopropanol–HCl–water (68/15·5/16·5).
   D.   *Sec.* Butanol saturated with water.
   E.   Isopropanol–NH³–water (7/1/2).

   (a)   Data of Alcantara and Wang.[16]
   (b)   Data of Weinblum and Johns.[17]
   (c)   Sample of 5-formyluracil courtesy of S. Y. Wang.
   (d)   For a description of the isomeric forms of the thymine dimer, see reference No. 18.
   (e)   Data of Smith.[19]

The other compound tested that is similar in behavior (but not identical) to the spore photoproduct is 5-hydroxymethyluracil.

Our chromatographic data allow us to conclude that the spore photoproduct is not identical with certain marker materials (Table 5) but does not allow us to establish the identity of the photoproduct.

B. What is the maximum yield of photoproduct? Since the spore photoproduct (R_f 0·35) shows no short wavelength reversal to thymine as compared to the cyclobutane-type thymine dimer (R_f 0·26), it is therefore possible that the spore product might not have a wavelength dependency for reaching a maximum yield of photoproduct as does the cyclobutane-type thymine dimer (for discussions of this phenomenon see references 20, 21). Early experiments (cited in Table 1) did in fact indicate that a much higher yield of thymine photoproduct was produced in spores (R_f 0·35 material) than in vegetative cells (thymine dimer, R_f 0·26) for a given dose of u.v.

For *Bacillus subtilis* 67·8 per cent of the thymines are adjacent to each other.[22] If after very high dose of u.v. (2537Å) the yield of the spore photoproduct (R_f0·35) plateaued at this value it would be good indirect evidence that the spore photoproduct was a dimer of

thymine. The results of experiments to measure the maximum percentage of the thymine converted to photoproducts versus dose of u.v. are given in Fig. 1. The yield of spore photoproduct ($R_f 0.35$) plateaued at a value of 28·4 per cent. The maximum yield of cyclobutane-type thymine dimer was about 2·6 per cent. This adds up to 31 per cent
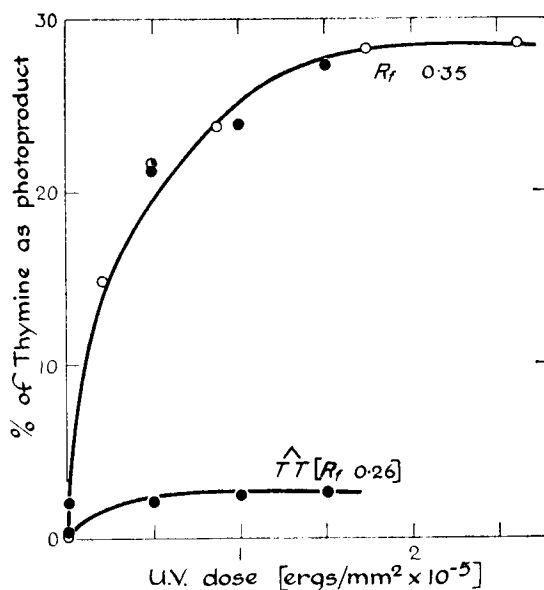

Fig. 1. Dose–response curves for the production of photoproducts of thymine in DNA of u.v.–irradiated spores of *Bacillus subtilis*.

The thymine–2–C–14 labeled spores discussed in the footnote to Table 4 were used here.

The different symbols indicate separate experiments. $\hat{TT}(R_f\ 0.26)$ represents the Type I cyclobutane-type thymine dimer. The R_f values are for the solvent used in Table 1.

which is only about half of the theoretical limit for the dimerization of thymine in single strands of DNA in *B. subtilis*. We can therefore offer no hypothesis concerning the structure of the spore photoproduct based upon these data alone. Donnellan and Setlow[7] reported a 31 per cent yield of this photoproduct in *B. megaterium* after a dose of 5×10^4 ergs/mm² at 2650Å.

C. Determination of molecular size by chromatography on Sephadex G-10. We had observed that a distinction between thymine (molecular weight 126) and the cyclobutane-type thymine dimer (molecular weight 252) produced when thymine was irradiated in frozen solution could be made on the basis of their behavior on a short column of molecular sieve material Sephadex G-10 (Fig. 2). The thymine dimer emerged first from the column with the peak centered at about tube No. 30 while the thymine was retarded by the column and emerged at tube No. 40. The Type III cyclobutane-type thymine dimer also emerged at tube No. 30.[19] When the spore photoproduct ($R_f 0.35$) was submitted to the column, it emerged at tube No. 36, which is about half way between the value for the monomer and the dimer. These results are therefore inconclusive in predicting the precise molecular weight of the spore photoproduct.
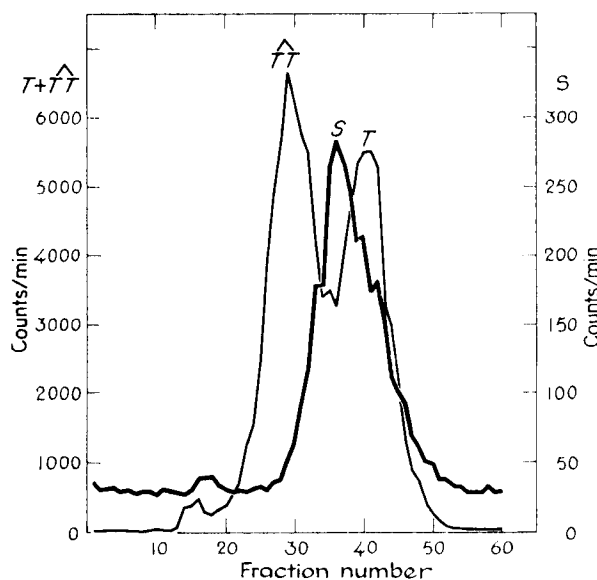
FIG. 2. Separation of photoproducts on a column of Sephadex-G10.
A column (0·8 × 35·5 cm) of Sephadex-G10 (40–120μ) was used. The samples were dissolved in and the column was equilibrated with water. 0·5 ml samples were collected every 2 min. T stands for thymine; $\hat{TT}$ for the cyclobutane-type thymine dimer produced when T is u.v. irradiated in frozen solution; and S for the spore photoproduct at R_f 0·35 (See Table 1).

DISCUSSION

The photochemistry of thymine in the DNA of *B. subtilis* spores is markedly different from that for vegetative cells. The chief photoproduct of thymine produced in vegetative cells is the cyclobutane-type thymine dimer. Very little of this type of thymine is produced in u.v. irradiated (2537Å) spores but another photoproduct of thymine is produced in high yield (maximum of about 28 per cent of the total thymine residues).

Experiments to establish the identity of this unique photoproduct were attempted but the structure still remains unknown. The cyclobutane-type thymine dimer can be distinguished from thymine by its behavior on a column of molecular sieve material, Sephadex G-10. The spore photoproduct, however, eluted about half way between the monomer and the dimer. The spore photoproduct is stable to reirradiation (2537Å) in solution in contrast to dimers of the cyclobutane-type. The fact that the spore photoproduct is isolated after the spores have been hydrolyzed in trifluoroacetic acid at 155°C for 60 min speaks for its stability to acid. The alkaline stability of the photoproduct has not been investigated.

The compounds that behave most similarly to the spore photoproduct in several solvents on paper chromatograms are 5-hydroxyuracil and 5-hydroxymethyluracil. It cannot be the former compound since the photoproduct is also formed when thymine–methyl-C-14 is used to label the spores. There appear to be sufficient differences in R_f values in at least two solvent systems to demonstrate a lack of identity between the spore photoproduct and 5-hydroxymethyluracil. The striking similarity in chromatographic properties between the spore photoproduct and these two hydroxylated derivatives, however, suggest that the spore photoproduct may be a partially oxidized derivative of thymine.

The cyclobutane-type dimer has been shown to be of biological importance in the u.v. inactivation of cells (for discussions of this see references 20, 21). It has been suggested that the greater resistance of spores to killing by u.v. as compared to vegetative cells may be due to the fact that little of this dimer is produced in irradiated spores, however, the high yield of this new thymine photoproduct implies either that it does not interfere with DNA synthesis or that the spores have a very efficient repair mechanism for this photoproduct[6]. This photoproduct is apparently lost from the spores during germination,[6] but does not appear in the acid soluble fraction.[8] If the spore photoproduct were repaired *in situ* and if this mechanism were more efficient than the "cut and patch" repair mechanism[23-25] shown to be operative on thymine dimers, this might explain the greater u.v. resistance of spores. However, if the new photoproduct is repaired by the same mechanism or with the same efficiency as the cyclobutane-type thymine dimer, then it is hard to see how the formation of this photoproduct in lieu of the cyclobutane-type thymine dimer could explain the u.v. resistance of spores.

E. coli cells are more sensitive to killing by u.v. when irradiated in frozen solution than they are when irradiated at room temperature[26,27]. Although the total amount of thymine altered by a given dose of u.v. is essentially the same whether the cells are irradiated at room temperature or at $-79°C$, there is a decrease in the amount of cyclobutane-type thymine dimer formed with a concomitant appearance of a material that is refractory to reirradiation in solution and which behaves chromatographically similar to the spore photoproduct.[27] If the spores are more resistant to killing by u.v. because of the formation of this photoproduct in lieu of the cyclobutane-type thymine dimer, then it is improbable that *E. coli* cells would be more sensitive to killing by u.v. as a consequence of the formation on this compound. The increased sensitivity of frozen *E. coli* cells to killing by u.v. has been correlated with an increase in the amount of DNA crosslinked to protein.[27]

The cell wall of a spore probably swells during germination and thus permits a better equilibration of the interior of the cell with exterior water. Except for hydration the physical state of spore DNA should not be altered by germination in the presence of chloramphenicol since this treatment prevents DNA replication.[9] The photochemistry of the DNA thymine of spores germinated in the presence of chloramphenicol is very similar to that of normal vegetative cells, and it suggests that the unique photochemistry of spores may involve a difference in the hydration of DNA.

The photochemistry of isolated DNA irradiated in solution is very different from that of DNA irradiated dry but is similar to that of DNA in vegetative cells. This suggests that the DNA within vegetative cells is thoroughly hydrated. Since the photochemistry of the DNA of spores differs markedly from that of vegetative cells or DNA in solution but is more similar to that of isolated DNA irradiated as a dry film, it suggests that the DNA within a spore is in a dry, compressed state. However, the yield of new photoproduct in dry DNA never reaches the yield that it does in spores, so dryness alone cannot explain the unique photochemistry of spores.

The DNA of spores that have been germinated in the presence of chloramphenicol does not act as a primer for DNA polymerase until the spores have been treated for a short time with deoxyribonuclease. We had hoped to explain this lack of priming activity on the basis that some unique physical state of the DNA had first to be changed. Recognizing that the physical state of the DNA has a marked effect on the photochemistry of DNA thymine[1-3] we had expected to see a change in the photochemistry of the spore DNA only after the deoxyribonuclease treatment. The physical state of the DNA that regulates

the formation of the cyclobutane-type thymine dimer between adjacent thymines in a single strand of DNA was not further altered after germination in chloramphenicol by a subsequent treatment with deoxyribonuclease. Our data therefore shed little light upon the problem of the priming activity of spore DNA for DNA polymerase.

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