Thymine Dimers and Inhibition of DNA Synthesis by Ultraviolet Irradiation of Cells

Abstract. *Measurements of the inhibition of DNA synthesis in bacteria and the formation of thymine dimers by ultraviolet irradiation of the cells indicate that one thymine dimer per 350-μ strand of DNA acts as a block to further synthesis. In a sensitive strain of* Escherichia coli *the blocks are permanent. In a resistant strain the blocks are only temporary but recovery of synthesis is not the result of splitting dimers.*

Ultraviolet radiation prevents colony formation in one strain of *Escherichia coli,* but has relatively little effect on the colony-forming abilities of another strain (*1*). We have now found that this type of radiation also has very different effects on DNA synthesis in these two strains. A strain which is sensitive to radiation, *E. coli* B_{s-1} (*2*), shows a marked inhibition of DNA synthesis when irradiated with doses that inhibit colony formation by 50 percent, but synthesis in resistant cells, *E. coli* B/r (ORNL), is only temporarily inhibited by much higher doses that do not prevent colony formation. It is known that the effects of ultraviolet irradiation on both colony formation and DNA synthesis may be reversed by the action of visible light (*3*), and that a photoreactivating enzyme preparation from yeast splits thymine dimers (*4*), that is, dimers between adjacent thymines in polynucleotide chains (*5*). Because the biological effect of the enzyme preparation does not seem to result from changes in any other photochemical products (6), it is a reasonable assumption that a large part of the inhibition of DNA synthesis that is observed following ultraviolet irradiation results from the formation of thymine dimers in polynucleotide chains. Support for this assumption comes from the observation that thymine dimers inhibit DNA synthesis in a polymerase system in vitro (*7*). Our data on DNA synthesis in bacteria indicate that a dimer acts as a block for DNA synthesis in vivo and that radiation-resistant cells have mechanisms for repairing or getting around these blocks, whereas sensitive cells do not. Chromatographic data show that the splitting of thymine dimers is not among the molecular events that lead to the recovery, in the dark, of DNA synthesis in resistant cells.

The synthesis of DNA in bacterial cultures was followed by measuring the incorporation of thymidine-methyl-H^3 (H^3TdR) into cells (*8*). Cultures growing exponentially at approximately 2×10^8 cells/ml in M9 medium (*8, 9*), supplemented with 0.25 percent casamino acids, were diluted tenfold into cold M9 medium and exposed for known times to monochromatic ultraviolet radiation of known intensities (about 5 erg/mm² per second). For photoreactivation, cells in M9 medium minus glucose, at 24°C, were exposed to 4000 Å radiation at an intensity of 10^3 erg/mm² per second. Following irradiation, glucose, casamino acids, adenosine, and H^3TdR were added to the culture and the suspension was incubated at 37°C. At various times, samples of the culture were pipetted onto stainless steel planchets that were subsequently washed so as to remove all the acid-soluble radioactivity (*9*). The planchets were counted in a gas-flow Geiger counter.

Labeled cells to be used for the chromatographic determination of thymine dimers were prepared by growing cultures in the radioactive medium for approximately five division times and resuspending them, following three centrifugations and washings, at 5×10^7/ml in nonradioactive medium. After irradiation, samples of between 1 and 5×10^7 cells were collected by centrifugation and hydrolyzed in formic acid; the hydrolysate was chromatographed in two dimensions (n-butanol-water followed by ammonium sulfate-sodium acetate-isopropanol) on Whatman No. 1 paper to separate thymine and the thymine dimers. Thymine moves ahead of the dimer in the first solvent and behind it in the second (*10*). The radioactive regions, located by ultraviolet absorption of nonradioactive markers, were cut out (see Table 1), eluted with water, and counted in a scintillation counter in which a dioxane-naphthalene scintillator was used (*11*). Net counts in the dimer region had a statistical fluctuation of 5 to 10 percent. The counting rate in the dimer region for cultures that were not irradiated was ≤ 0.005 percent of that in the thymine region.

Figure 1*a* shows the effects of various doses of ultraviolet radiation on DNA synthesis in *E. coli* B_{s-1}. (The dose at 2650 Å that permitted 30 percent of the colonies to form was about 2 erg/mm².) Both the time at which synthesis ceased and the level of DNA

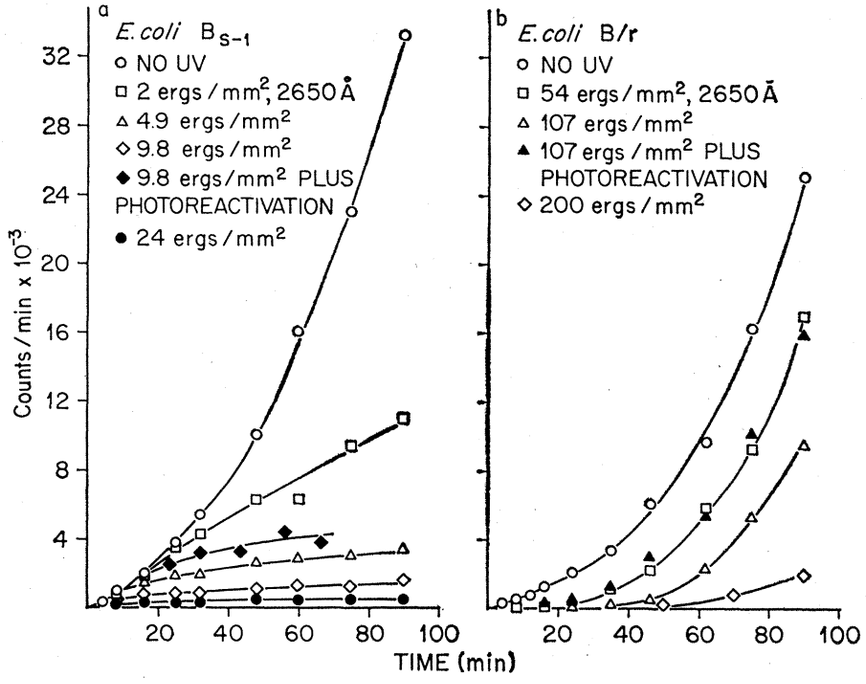

Fig. 1. The incorporation of H^3TdR into ultraviolet-irradiated (2650 Å) and photoreactivated (4000 Å, 6×10^3 erg/mm²) cultures of *E. coli*. Each point represents the average of two 10-μl samples from 220-μl incubation mixtures containing at zero time: H^3TdR (2 μg/ml, 6 c/m*M*), adenosine (200 μg/ml), and bacteria (~ 2×10^7/ml) in M9 medium supplemented with casamino acids. Division in unirradiated cultures took 34 minutes. Photoreactivating exposure had no effect on cultures that were not irradiated.

synthesis attained by an irradiated culture decreased with increasing dose. Photoreactivating light decreased the effect of irradiation. A model that would fit these kinetic data would be one in which DNA polymerization along a "primer" took place until it was blocked (by a thymine dimer), at which point it would stop. The more blocks there were, the sooner polymerization would stop and the less synthesis there would be. Such a model would fit our data if there were about one block per synthesizing unit for every 2 erg/mm^2. Chromatographic data (given herein) indicate that 2 erg/mm^2 produce one dimer in a DNA strand 350 μ in length —a length equal to half of that of a replicating DNA unit of *E. coli* (*12*). Thus we infer that, in bacteria, a dimer represents a block to DNA synthesis—all of which takes place along long units.

Figure 1*b* shows the results obtained when the radiation-resistant strain, *E. coli* B/r was irradiated. At the doses used there was only a small effect on the colony-forming ability of these bacteria. After irradiation there was an almost complete inhibition of DNA synthesis, but later, the bacteria recovered their ability to synthesize DNA; they were somehow able to circumvent or repair the blocks. When irradiated cells were placed in a medium containing no nutrient (M9 medium minus glucose) the lag in DNA synthesis decreased. It appears that the resistant cells might have an enzyme system which is able to split the thymine dimers in the dark.

Thymine dimers in cells were measured at various times after irradiation. Representative analytical data are given in Table 1. The difference between sensitive and resistant strains is not the result of different efficiencies of dimer production since, within the overall absolute accuracy, both strains show the same fraction of dimers (Table 1, entries IV, V). Thymine dimers are not split in appreciable numbers during the time corresponding to the resumption of DNA synthesis (entries I, II, III). For the photoreactivation exposure used in Fig. 1, the number of thymine dimers changes by a factor of about 0.5 (entry VI), a factor whose correspondence with the dose-reduction factor for DNA synthesis shown in Fig. 1, *a* and *b* is further evidence for the blocks to synthesis being dimers. A similar dose-reduction factor is found for colony formation. The ratio of activity in dimers to that in thymine for *E. coli* B_{s-1} exposed to 200 erg/mm^2 2650 Å is 0.097 percent (entry V). Since the number of dimers is proportional to dose (entries I, II, IV), by interpolation, the number of dimers per thymine for 2 erg/mm^2 would be 5×10^{-6}. The number of dimers per nucleotide would then be approximately 10^{-6}. Taking the distance between nucleotides as 3.4 Å, we arrive at the result that 2 erg/mm^2 of 2650 Å produces one dimer in approximately 3.5×10^6 Å (350 μ) of a DNA strand.

Since thymine dimers are not split in the resistant cells, we are investigating other molecular mechanisms that may account for the resumption of DNA synthesis, such as the possibility of polymerizing at a slow rate around a block or that the dimer is cut out of the DNA chain by nucleases (*13*) and is replaced by two thymines. The first possibility seems to exist in a system which is in vitro (*7*). The second is consistent with the postulated mechanisms concerning host-cell reactivation and ultraviolet reactivation of bacteriophage assayed on irradiated and unirradiated sensitive and resistant mutants (*14*). These mechanisms involve the interplay of two enzyme systems: nucleases that magnify the initial effects of ultraviolet radiation, and reactivating enzymes that tend to increase survival after irradiation.

Table 1. The distribution of radioactivity in *E. coli* between thymine and thymine dimer regions. Sections of the chromatograms containing dimers were cut into 1-cm strips, counted for 10 minutes, and background (30 to 40 count/min depending on the experiment) was subtracted. The resulting activities were plotted against position and the points outside the dimer region were connected by a smooth curve that defined non-dimer activity in the dimer region. The net activity in dimers was taken as the difference between the resultant activity and non-dimer activity. The total radioactivity remained constant during the various treatments of cells after irradiation.

Treatment*	Counts above background per 10 min			Thymine (count/min)	Dimer/thymine (%)
	Dimer region	Non-dimer activity	Net dimer activity		
Entry I. E. coli *B/r, irradiated at 2650 Å, 100 erg/mm²*					
No ultraviolet radiation	75	65	10	19,500	0.005
Irradiated only	270	70	200	29,300	0.069
Irradiated and held 30 min	480	90	390	54,100	0.072
Irradiated, held 30 min, and grown 225 min	500	130	370	60,300	0.061
Entry II. E. coli *B/r, irradiated at 2650 Å, 100 erg/min²*					
No ultraviolet radiation	350	380	−30	221,500	−0.001
Irradiated only	1560	340	1220	216,600	0.057
Irradiated and held 30 min	1570	160	1410	194,200	0.073
Irradiated, held 30 min, and grown 90 min	2160	360	1800	251,400	0.072
Irradiated, held 30 min, and grown 175 min	970	160	810	150,700	0.054
Entry III. E. coli *B/r, irradiated at 2805 Å, 200 erg/mm²*					
No ultraviolet radiation	130	100	30	92,100	0.003
Irradiated only	790	270	520	73,200	0.071
Irradiated and grown 90 min	490	130	360	50,200	0.072
Irradiated and grown 120 min	580	170	410	60,000	0.068
Irradiated and grown 190 min	400	60	340	54,000	0.063
Entry IV. E. coli *B/r, irradiated at 2650 Å, 200 erg/mm²*					
Irradiated only	660	110	550	43,900	0.125
Entry V. E. coli B_{s-1}, *irradiated at 2650 Å, 200 erg/mm²*					
Irradiated only	560	70	490	50,400	0.097
Entry VI. E. coli B_{s-1}, *irradiated at 2805 Å 400 erg/mm²*					
Irradiated only	1470	300	1170	88,600	0.132
Irradiated and photoreact.† 3×10^5 erg/mm²	640	190	450	49,900	0.090
Irradiated and photoreact. 6×10^5 erg/mm²	690	310	380	54,100	0.070

*Cells were held in a non-nutrient medium, M9 minus glucose, at 37°C. Cells were grown in nutrient medium, M9 plus casamino acids, at 37°C. †Photoreactivation was affected at 4000 Å.

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References and Notes

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Calcium: Unusual Sources in the Highland Peruvian Diet

Abstract. *A dietary survey conducted in the southern highlands of Peru revealed two important sources of dietary calcium not previously reported. Mineral and ash calcium ingested as a food spice, and along with coca, raises the calcium intake from the low figures recorded by standard nutritional surveys to a more substantial level.*

The highland area of Peru has been specified in dietary reports as an area where the inhabitants have a low calcium intake (*1*), and studies of growth seem to support this finding (*2*). However, during our recent studies of nutrition in the southern highlands of Peru, we discovered several unusual sources of calcium which seem to provide for most people a more than adequate intake of this necessary mineral.

The results from some dietary surveys made in non-Western cultures are open to serious question because of the highly divergent dietary patterns which are encountered. These divergent patterns often mean that surveys in which standardized techniques derived from Western culture are used may cause important nutritional sources to be missed. As was clearly shown by the work in Mexico (*3*), many unexpected sources of essential nutrients may be revealed when detailed analyses of native diets are undertaken. Therefore, when we made a nutritional survey in the highlands of Peru during July 1962, a special effort was made to determine whether the techniques previously used in this area were, indeed, uncovering all sources of calcium intake.

The survey was made in the vicinity of Nuñoa, in the highest inhabited zone of the Peruvian Andes which ranges from an altitude of 4000 to 5000 m. The inhabitants are Quechua Indians, and the survey was conducted in the native-controlled community or *ayllu*, and in privately owned haciendas which lie outside of the small district capital. All the data were collected by one of us (R.B.M.) with the assistance of two Peruvian university students who were natives of the district. Spanish and Quechua were used depending on the language fluency of the respondent.

The method, applied to 39 households, was a modified 1-day recall technique in which food consumption for a single day was either observed and weighed, or the household cook was asked to indicate an equivalent of food stuffs which were then weighed. Individual intakes were calculated by dividing the quantity by the number of individuals over 3 years of age who ate within the household.

It was found that in this pastoral area of the Department of Puno, the intake of calories and other nutrients estimated by this method was greater than has been reported for other highland areas. Compared to other groups, calcium intake through regular food sources was high, approximately 430 mg per day. A study of yearly food patterns by means of questionnaires suggests that an even higher intake would have been recorded if the survey had been made during the period from December through March, since the Nuñoa indigenes reported that they consume large quantities of milk and cheese at that time.

In addition to calcium from the usual food sources, observation led to the belief that significant calcium was also being obtained from items not ordinarily considered in dietary surveys. Three items were chosen for further analysis: (i) an earth called *cal* or *catahui*, (ii) an earth called *"cha'qo,"* and (iii) an ash substance called *llipta.* Intake of these substances was recorded and a chemical analysis of samples was made at the Foods and Nutrition Laboratory, Pennsylvania State University.

The *cal* is prepared by burning calcium-containing rocks and grinding the remains into a fine powder. The powder is most often eaten mixed with *quinua* or *cañihua* in a porridge called *catahui lahua. Catahui lahua* is eaten at least a few times a week throughout the year by most of the Nuñoa households. A typical individual intake of *cal* at a meal would be about 5 grams. Averaged over a week, the daily intake is, therefore, within a range of 1 to 3 g. Analysis of two samples showed the *cal* to be primarily calcium carbonate, with a calcium content of 36.3 percent in one sample and of 42.0 percent in the other.

Cha'qo is a clay substance which is used in a fine water suspension as a sauce for potatoes. It is an aluminosilicate containing no calcium and is apparently without great nutritional value, although it does contain some iron.

To form *llipta,* the ashes of the stalks of two local grains, quinua and cañihua, are mixed with water to form a paste which is dried in the sun into small black cakes. A pinch from the cake is taken with every chew of coca (*4*). The average daily intake of coca is variable, but ranges from about 25 to 75 g and about 2 to 4 g of *llipta* is taken with this quantity of coca. Adult indigenes in the southern highlands chew coca with *llipta* nearly every day throughout the year. The *llipta* analyzed contained about 12 percent calcium as well as considerable magnesium. In the northern highlands there is a similar intake of coca, but *cal* rather than *llipta* is used in combination with the coca (*5*).

Two of these substances, therefore, contribute significant usable calcium to the diet of the highland adult. The *cal* eaten in this area contributes 300 to 1200 mg daily, while the daily *llipta* ingested contains somewhere between 200 and 500 mg. Although women and men in our sample used coca with the same frequency, children under 15 do not regularly chew coca and so do not ingest *llipta.* However, they do eat *catahui lahua* and with the same frequency as the adults, even though actual quantities may vary.

When calcium from these sources is added to that obtained from normal foods, it appears that natives of our study area, and perhaps much of the southern highlands, meet or exceed most of the calcium intakes recommended in the United States. Even in the northern highlands where *catahui lahua* is not reported, the *cal* chewed with coca, and perhaps coca itself (*6*) may constitute significant contributions to calcium intake. If *cal* is used in quantities equivalent to *llipta,* it alone