TRYPTOPHAN PHOTOPRODUCT AS A GENETIC PROBE: EFFECTS ON BACTERIA

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SUMMARY

Recombinationless (rec) mutants of bacteria are sensitive to visible and near-ultraviolet wavelengths of light. In addition, these mutants are sensitive to a tryptophan photoproduct that results from irradiation of this amino acid by 280-365 nm wavelengths. The physical and biological damages are different from those produced by 254 nm UV. Both the longer wavelengths and the tryptophan photoproduct are mutagenic and influence the genetic recombinational process in bacteria. Since the natural environment includes an abundance of both free tryptophan and sunlight, the relevance of the effect of these agents is provocative.

INTRODUCTION

The classical paper by STADLER and UBER (1942) emphasized the mutational effect of 254 nm ultraviolet radiation. Hidden within the same paper is the observation that longer wavelengths may also cause mutations, although the frequency may appear to be diminished. However, the abundance of solar radiation with emissions above 300 nm provokes our inquiry as to the genetic significance of these longer wavelengths, especially in evolutionary and ecological terms.

The importance of visible and near-visible light (V-NUV) to other biological functions are under constant study by scientists (i.e., photosynthesis, taxis, vision, rhythm, tropism, behavioral response, and differentiation. See Figure 1). Specific to this discussion, we need to examine whether constant bombardment by V-NUV has ultimate genetic consequences. If so, we will need to identify the photo-receptor(s), the intermediate metabolic steps, and the specific chromosomal damage that is the basis of the subsequent genetic manifestation.

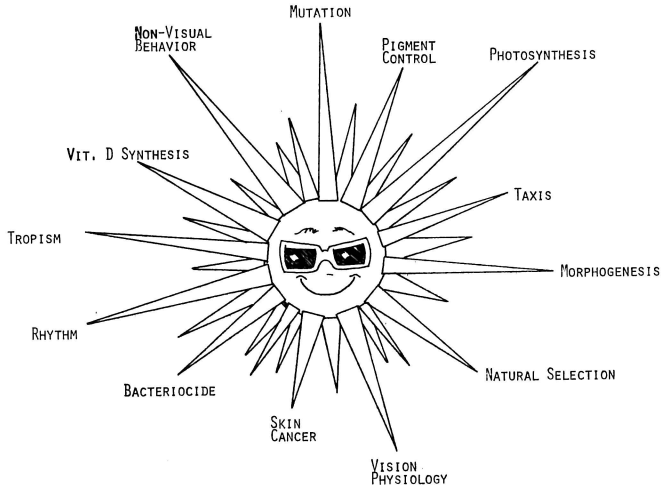

FIGURE 1. Biological phenomena affected by sunlight.

EVIDENCE THAT WAVELENGTHS ABOVE 300 NM ACT AT CHROMOSOMAL LEVEL

Radiation biology, with its voluminous literature, has recorded many experiments in which mutation and other genetic damage by visible and near-UV light have been implicated (EISENSTARK 1971). However, more direct proof of chromosome alteration came from our observation that certain mutants of *Salmonella typhimurium* are very sensitive to visible light; they are also recombinationless (*rec*) mutants that are unable to repair DNA damage. Wildtype and other UV-sensitive mutants are not sensitive to visible light. These *rec* mutants are also sensitive to a variety of toxic agents, but when carefully sorted out, those agents to which *rec* mutants are differentially far more sensitive, are also carcinogens and mutagens suspected of producing chromosomal damage.

Although the sensitivity of *rec* mutants to V-NUV appeared to be an exciting observation, before committing our resources to extensive experiments, we deliberated as to whether we might be observing an artifact.

EVIDENCE THAT V-NUV PRODUCES A LETHAL EFFECT, AND THAT NO 254 *nm* LEAKAGE CONTAMINATES OUR EXPERIMENTS

Perhaps the best evidence of the reality of the V-NUV effect is that V-NUV light produces biological alterations that are very different from those produced by 254 nm. This information is summarized in Table 1.

TABLE 1. Differences Between UV (254 nm) and V-NUV (above 300 nm) Effects on Bacteria

UV	V-NUV	Reference
Small O_2 demand for death	Absolute O_2 demand for death	EISENSTARK 1970
Death in stationary or growth phase	Death in growth phase only	EISENSTARK 1970
Small delay in division	Severe delay in division	JAGGER 1972
DNA degradation in recA mutants	No DNA degradation	FERRON *et al.* 1972
Pyrimidine dimer formation	No dimers at lethal doses	FERRON 1971
Phage multiply in UV-killed host cells	Phage multiplication halted at low (non-lethal) doses	FERRON *et al.* 1972

As may be seen from Table 1, there are striking differences between UV and V-NUV biological effects, at several levels, including DNA alterations and gross biological changes. Also, careful studies have been performed with monochromatic sources of light and interference filters (MACKAY 1971) to produce V-NUV genetic damage. These procedures reduce the possibility that our light source might be contaminated with 254 nm UV.

DNA DAMAGE PRODUCED BY V-NUV

Numerous reports indicate that the major (but not the sole) damage produced by 254 nm is pyrimidine dimerization. The observation that *S. typhimurium hcr* mutants, (which are unable to excise dimers) are not very sensitive to V-NUV (EISENSTARK 1970), suggests that the major DNA damage by V-NUV is not dimer formation. However, it should be noted that dimers may be formed by large doses of 365 nm irradiation (TYRRELL 1973). Also, *Escherichia coli* cells killed by 365 nm can be photoreactivated (BROWN and WEBB 1972), which is further evidence that dimers may be formed, as well as other damages.

We do not yet know the major molecular alteration produced by V-NUV, nor do we know the precise substrates that *rec*+ proteins can recognize (HANAWALT 1972). The work of FERRON (1971) emphasizes the difference between UV and V-NUV; at lethal doses of V-NUV, neither dimers nor single strand DNA breaks were found to be prevalent.

GENETIC DAMAGE BY TRYPTOPHAN PHOTOPRODUCT (TP)

Another discovery in our laboratory permitted us to view the genetic effects of V-NUV in a fresh manner. Recombinationless mutants may be killed, not only by direct V-NUV, but also by merely adding cells to media that has been exposed to light (WEBB and LORENZ 1972, YOAKUM and EISENSTARK 1972), although the cells themselves are kept in constant darkness.

What ingredient of the medium is responsible for the toxic photoproduct? After screening a large number of components, we found that tryptophan alone, upon exposure to V-NUV, is lethal to our bacterial strains. No other amino acid, sugar, or inorganic salt has this effect (YOAKUM and EISENSTARK 1972).

Also important, TP is only effective against *rec* mutants; it does not harm wildtype or other UV-sensitive mutants. This has led us to a search of the precise genetic damage produced by TP.

While our findings deal only with free tryptophan, the role of photoactions on tryptophan within enzyme molecules should not be overlooked (VOLKERT and GHIRON 1973).

STATUS OF SEPARATION AND IDENTIFICATION OF ACTIVE TP

An important contribution will be the utilization of purified TP in experiments. Unfortunately, this purification has not yet been achieved. Some of the chemical properties of TP have been ascertained (YOAKUM 1971) and numerous attempts have been made to separate active TP via thin layer chromatography and to assay by gas chromatography and mass spectroscopy. The ability to obtain distinct data is handicapped by the fact that a mixture of many photoproducts are obtained after irradiation of tryptophan. Nevertheless, with the aid of colleagues at UMC (Drs. McCormick, Chemistry Department, and Ghiron, Biochemistry Department), we are now hopeful that the active TP can be separated and identified, and that we will be able to examine the interaction of purified TP with bacterial chromosomes.

ACTION SPECTRUM FOR CONVERSION OF TRYPTOPHAN TO TP

To determine the wavelengths that would convert L-tryptophan to the toxic TP, samples were irradiated with monochromatic light, utilizing a xenon arc lamp in a Schoeffel housing coupled with a Bausch and Lomb 500 nm diffraction grating monochromator, and with Corning glass cutoff filters. After irradiation, samples were tested for toxicity of our *recA S. typhimurium* strain.

Our results showed that there is a definite peak of toxic activity when samples are exposed to 290 nm (although the L-absorption peak is 280 nm), but the curve is skewed into the near-UV region, up to 370 nm (Figure 2). Interestingly, while L-tryptophan has some absorption in the 240-260 nm range, no detectable TP is formed at these lower wavelengths.

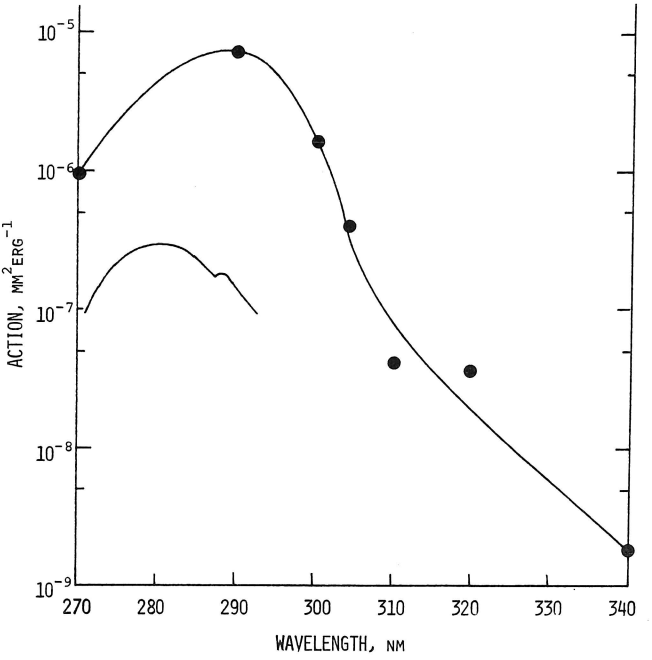

FIGURE 2. Action spectrum of toxicity for irradiated L-tryptophan (from YOAKUM 1971).

Top curve: Following exposures of L-tryptophan to various wavelengths of light, samples were tested for toxicity to *recA S. typhimurium*. Data were normalized to 1% survival level.

Bottom curve: Absorption spectrum for L-tryptophan. Note that absorption peak (280 nm) is lower than toxicity peak (290 nm).

MUTATION BY V-NUV AND BY TRYPTOPHAN PHOTOPRODUCT

Since the initial experiments of STADLER and UBER (1942), several studies have demonstrated that wavelengths above 254 nm produce mutations (KUBITSCHEK 1967, WEBB and MELINA 1967, WEBB 1972), including those in the V-NUV range. This, of course, is evidence that these longer wavelengths produce DNA alterations, although the final change may result indirectly from a chain reaction.

To test whether TP is also mutagenic, YOAKUM (1971) utilized the chemostat technique of KUBITSCHEK (1967) and scored for induction of *E. coli* resistance to T5 phage. YOAKUM's results (Table 2) indicate that TP is mutagenic, and further confirmatory experiments are underway.

TABLE 2. The Appearance of T5 Resistant Mutants in a Continuous Culture of *E. coli* B/r/t upon Exposure to Irradiated L-tryptophan (abbreviated from Yoakum 1971)

Time (hrs)	Viable Count ($\times 10^7$)	T5 Resistant Mutants	T5 Resistant Mutants/10^7 Cells
0	4.4	1.5×10^2	46
2	2.6	2.8×10^3	864
5	4.0	4.6×10^3	1.42×10^3
7	1.8	9.9×10^3	3.05×10^3
18	3.8	2.12×10^4	6.54×10^3
24	2.6	2.75×10^4	8.49×10^3
46	4.0	3.88×10^4	1.20×10^4

The listings for "viable counts" and "T5 resistant mutants" are the averages of four platings. The number of T5 resistant mutants per 10^7 cells was determined using the average of all viable counts and the number of T5 resistant cells at each time.

INFLUENCE OF TP ON GENETIC RECOMBINATION

There are several related reasons for testing whether TP might influence recombinational events. First, if the site of TP action is the chromosome itself, an effect on genetic

recombination might be anticipated. Second, if some of the enzymes involved in DNA repair and in recombination have dual and interchangeable functions, one might anticipate that a TP assault on the chromosome would enlist these enzymes into the repair process, and make them less available for recombination events. This latter point is somewhat obscured by the fact that the recombinational event itself may be the mode of some DNA repair (HANAWALT 1972 for review). Nevertheless, it was felt that an examination of the influence of TP on recombination might give us some insight into its mode of action.

TABLE 3. Influence of Tryptophan-Photoproduct (TP) on Recombination in Bacteria

System	Recombination frequency/10^8 cells		
	In Tryptophan	In TP	Result
E. coli Hfr x F-455 *pro*$^-$ *leu*$^-$	8×10^5	0.5×10^5	16X decrease
T4 phage rII x rII	9×10^5	2.8×10^5	3.2X decrease
S. typhimurium transduction of *his*	6.9×10^1	1.7×10^2	2.5X increase
Induction of *S. typhimurium* phage P22	5.1×10^6 PFU/ml	3.5×10^7 PFU/ml	7X increase
Induction of *E. coli* phage lambda	1.8×10^6 PFU/ml	8.3×10^6 PFU/ml	4.6X increase
B. subtilis transformation	5.3×10^3	4.9×10^3	no change

As may be seen (Table 3) *E. coli* conjugation, T4 phage recombination and phage induction were reduced as anticipated, but *S. typhimurium* responded with an enhancement of recombination.

In numerous experiments of *E. coli* conjugation (LANDA and EISENSTARK 1973), the number of recombinants in *E. coli* Hfr x F-crosses, are always reduced when recipient cells are treated with TP. Additional experiments support the view that the interference is at the chromosomal level, and there is neither an interference with cell-to-cell pair formation, nor with the transfer of DNA from male to female, nor a

delay of DNA synthesis is involved. The details of these experiments will appear elsewhere.

The transduction of *his* recipient *S. typhimurium* with P22 from *his*$^+$ donors gave results that are not consistent with *E. coli* conjugation and the T4 phage recombination data. It is difficult to offer a satisfactory explanation until we have more precise details of molecular interchange in these different recombinational processes.

The increase of transductants after treating recipient cells with TP is reminiscent of the experiments by GAREN and ZINDER (1955) in which pretreatment of donor phage with low doses of 254 nm UV (but not ^{32}P) increased the frequency of transduction. ADYE (1962) studied the same phenomenon with nitrous acid-treated P22 phage, and BENZINGER and HARTMAN (1962) did detailed experiments with low doses of UV, but neither offered an explanation, at the molecular level, to account for this increase in recombination. While these agents may produce an increased number of nicks in the DNA, it is puzzling that this increase would occur in transduction but not in other forms of recombination.

INDUCTION OF PHAGES P22 *(S. typhimurium)* AND LAMBDA *(E. coli)*

As may be seen (Table 3) TP is an excellent inducer of phage production in lysogenic cells of *S. typhimurium* and *E. coli*. This is true of a large number of mutagens and carcinogens. These facts and the abundant presence of tryptophan in cells support our contention that TP is an important biomolecule. Phage induction involves genetic recombination, but there remain some puzzles regarding precise mechanisms. In *recA S. typhimurium*, the absence of spontaneous induction of phage synthesis is particularly striking (EISENSTARK *et al.* 1969), although the exact relationship to failure in genetic recombination is obscure.

RELEVANCE

A large fraction of biological research has been devoted to 254 nm UV and X-ray studies. Recent observations of biological effects by V-NUV (above 300 nm), especially that of genetic damage, now brings new focus to this field.

In a current clinical practice, therapy for hyperbilirubinemia, newborn infants are exposed to high-intensity blue light (BEHRMAN and HSIA 1969). Are there other cellular effects, and are any of these damaging? Mutation and possible carcinogenesis by V-NUV need to be evaluated. Modern technology, of course, increases exposure to V-NUV wavelengths. Among other items, there is increased use of household and industrial fluorescent lamps, as well as blacklight (300-400 nm)

in the entertainment business (e.g., illumination of psychedelic posters). Also, blacklight lamps are used for suntanning.

In addition to V-NUV biological effects produced by artificial illumination, there is need for clearer understanding of the effects of sunlight, especially in human medicine (DANIELS 1964). Our research has emphasized effects on bacterial cells, but in at least one human disease, xeroderma pigmentosum, cells appear to be unable to excise pyrimidine dimers produced by sunlight damage (CLEAVER and TROSKO 1970, TAKEBA *et al* 1972), much like our *hcr* mutants of coliform bacteria. Sunburn and suntanning problems have received new attention with the knowledge that the drug psoralen can cause interstrand crosslinks in DNA when exposed to sunlight (COLE 1971, KARU and LINN 1972).

Our findings with tryptophan have aroused our curiousity as to the abundance of this amino acid in nature and any special roles it may have in biological phenomena (FINDLAY 1972, ROSE 1972). Particularly striking is its abundance in specific nerve cells where it is involved in serotonin metabolism, an important behavioral molecule (CARLSSON and LINDQVIST 1972). This information, coupled with the knowledge that light can penetrate through such tissues as the skulls of fish, birds and small mammals, leads to speculation on the interaction of tryptophan with light in behavioral responses. Especially interesting is the report by MENAKER (1972) in which he described non-visual light responses that trigger gonad development and the accompanying behavioral responses.

The recognition that eye pigment mutants of Drosophila lack an enzyme responsible for the breakdown of tryptophan (JACOBSON 1971) emphasizes a role of this amino acid in vision. The interaction of tryptophan with near-UV in cataract formation is also provocative (GROVER and ZIGMUND 1972).

In other areas of medicine, numerous examples of special roles for tryptophan metabolism and visual light effects may be cited, including vitamin D deficiencies, pellagra, and skin pigment formation (DANIELS 1972, ROSE 1972). While many additional items could be added to our list, the above are of sufficient importance to urge us to seek any possible generalities as to (1) how tryptophan metabolism fits into any genetic, evolutionary, or ecological scheme, and (2) whether the effect of sunlight on tryptophan is a significant factor in any of these schemes.

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LITERATURE CITED

ADYE, J. 1962 Effect of nitrous acid on transduction by Salmonella phage P22. Virology *18*:627-632.

BEHRMAN, R. D. and D. Y. HSIA 1969 Summary of symposium on phototherapy for hyperbilirubinemia. J. Pediatr. *75*:718-726.

BENZINGER, R. and P. E. HARTMAN 1962 Effects of ultraviolet light on transducing phage P22. Virology *18*:614-626.

BROWN, M. S. and R. B. WEBB 1972 Photoreactivation of 365 nm inactivation in *Escherichia coli*. Mutation Res. *15*:384-352.

CARLSSON A. and M. LINDQVIST 1972 Effect of L-tryptophan and some psychotropic drugs on the formation of 5-hydroxytryptophan in the mouse brain *in vivo*. J. Neural Transmission *33*:23-43.

CLEAVER, J. E. and J. E. TROSKO 1970 Absence of excision of ultraviolet cyclobutane dimers in xeroderma pigmentosum. Photochem. Photobiol. *11*:547-550.

COLE, R. S. 1971 Psoralen monoadducts and interestrand cross-link in DNA. Biochim. Biophys. Acta *254*:30-39.

DANIELS, F., JR. 1964 Man and radiant energy: solar radiation. Dill, D. B. (Ed.) Handbook of Physiology. Section 4: Adaptation to the Environment. American Physiol. Soc. William and Wilkins, Baltimore.

DANIELS, F. 1972 Photochemical effects of sunlight. Biophys. J. *12*:723-727.

EISENSTARK, A. 1970 Sensitivity of *S. typhimurium* recombinationless *(rec)* mutants to visible and near-visible light. Mutation. Res. *10*:1-6.

EISENSTARK, A. 1971 Mutagenic and lethal effects of visible and near-ultraviolet light on bacterial cells. Adv. Genet. *16*:167-198.

EISENSTARK, A., R. EISENSTARK, J. VAN DILLEWIJN, and A. RÖRSCH, 1969 Radiation-sensitive and recombinationless mutants of *Salmonella typhimurium*. Mutation Res. *8*:497-504.

FERRON, W. L. 1971 DNA abberations following irradiation of recombinationless *(recA)* mutants of *S. typhimurium*. Ph.D. dissertation, Kansas State University, Manhattan, Kansas.

FERRON, W. L., A. EISENSTARK, and D. MACKAY 1972 Distinction between far- and near-ultraviolet light killing of recombinationless *(recA) Salmonella typhimurium*. Biochim. Biophys. Acta. *277*:651-658.

FINDLAY, G. H. 1972 Tryptophan and light. VI Int. Congr. Photobiol., Bochum, W. Germany. Abstract no. 053.

GAREN, A. and N. D. ZINDER 1955 Radiological evidence for partial genetic homology between bacteriophage and host bacteria. Virology *1*:347-376.

GROVER, D. and S. ZIGMUND 1972 Coloration of human lenses by near-ultraviolet photo-oxidized tryptophan. Exp. Eye Res. *13*:70-76.

HANALWALT, P. C. 1972 Repair of genetic material in living cells. Endeavour, *31*(113):83-87.

JACOBSON, K. B. 1971 Role of an isoacceptor transfer ribonucleic acid as an enzyme inhibitor: Effect on tryptophan pyrrolase of *Drosophila*. Nature New Biol. *231*:17-20.

JAGGER, J. 1972 Growth delay and photoprotection induced by near-ultraviolet light. Res. Prog. in Org. Biol. and Med. Chem. Vol. 3, Pt. I:383-401.

KARU, A. E. and S. LINN 1972 Uncoupling of the RecBC ATPase from DNase by DNA crosslinked with psoralen. Proc. Nat. Acad. Sci. U.S. *69*:2855-59.

KUBITSCHEK, H. E. 1967 Mutagenesis by near-visible light. Science *155*:1545-1546.

LANDA F. and A. EISENSTARK 1973 The effect of L-tryptophan photoproduct on genetic recombination in bacteria. (abstract) Proc. Am. Soc. Microbiol., p. 73.

MACKAY, DONNA 1971 Action spectrum of lethality in a recombinationless *(recA)* mutant of *Salmonella typhimurium*. M. S. thesis, Kansas State University, Manhattan, Kansas.

MENAKER, M. 1972 Nonvisual light reception. Sci. Amer. *228*(3):22-29.

ROSE, D. P. 1972 Aspect of tryptophan metabolism in health and disease: A review. J. Clin. Pathol. *25*:17-25.

STADLER, L. D. and F. M. UBER 1942 Genetic effects of ultraviolet radiation in maize IV. Comparison of monochromatic radiations. Genetics *27*:84-118.

TAKEBE, H., J. I. FURUYAMA, Y. MIKI, and S. KONDO 1972 High sensitivity of xeroderma pigmentosum cells to the carcinogen 4-nitroquinoline-1-oxide. Mutation Res. *15*:98-100.

TYRRELL, R. M. 1973 Induction of pyrimidine dimers in bacterial DNA by 365 nm radiation. Photochem. and Photobiol. *17*:69-73.

VOLKERT, W. A. and C. A. GHIRON 1973 The destruction of tryptophanyl residues in trypsin by 280-nm radiation. Photochem. Photobiol. *17*:9-16.

WEBB, R. B. 1972 Photodynamic lethality and mutagenesis in the absence of added sensitizers. Biol. Med. Chem. Vol. 3, Part II: 511-530.

WEBB, R. B. and J. LORENTZ 1972 Toxicity of irradiated medium for repair-deficient strains of *Escherichia coli*. J. Bacteriol. *112*:649-652.

WEBB, R. B. and M. M. MALINA 1967 Mutagenesis in *Escherichia coli* by visible light. Science *156*:1104-1105.

YOAKUM, G. H. 1971 Photoxocity of near-UV irradiated L-tryptophan for recombinationless mutants of *Salmonella typhimurium*. M. S. thesis, Kansas State University, Manhattan, Kansas.

YOAKUM, G. and A. EISENSTARK 1972 Toxicity of L-tryptophan photoproduct on recombinationless *(rec)* mutants of *Salmonella typhimurium*. J. Bacteriol. *112*:653-655.

Dr. Eisenstark in a discussion group at the Symposium