Effect of Tryptophan Analogues on Reversion of a Tryptophan-requiring Strain of *Escherichia coli*

Two years ago, Witkin[1] reported experiments indicating that protein synthesis was essential for the 'fixation' of the mutagenic state induced by ultra-violet irradiation. More recently, Doudney and Haas[2] have shown that at least two processes are involved in the production of bacterial mutations by ultra-violet irradiation; the first process results in fixation of the mutation into the genome, the second involves a removal of the mutagen produced by the radiation. We would like to report experiments that indicate that protein synthesis is involved in the actual utilization or degradation of the mutagen produced by ultra-violet irradiation.

Washed broth cultures of a tryptophan-requiring mutant of *Escherichia coli* strain *B/r* (isolated in this laboratory) were used in these experiments. Reversion of this mutant to the prototrophic condition was determined by plating cell suspensions on Petri dishes containing minimal medium. Ultra-violet irradiated cells, irradiated in buffer, were incubated for 1 hr. at 35° in 10 ml. of liquid medium of the indicated composition contained in centrifuge tubes (Table 1), they were then collected, washed and re-incubated for 1 hr. as indicated after which they were plated on minimal medium *C*[3]. Survival was determined by plating on minimal medium *C* supplemented with tryptophan. The amino-acid mixture (AA) was prepared according to Witkin[1] and contained no tryptophan. DL-Tryptophan, DL-5-methyl tryptophan and DL-tryptazan were added to give a concentration of 40 μgm./ml. The tryptazan was a gift from Dr. H. R. Snyder.

It is readily apparent that we have obtained the effect reported by Witkin; a large increase in the number of prototrophs was obtained by incubation immediately following irradiation in a medium rich in amino-acids. It is also apparent that the 'mutagen' produced by ultra-violet is stable when washed cells are incubated in medium with no tryptophan added or when 5-methyl tryptophan is added (Table 1). The mutagen disappears, however, following incubation with DL-tryptazan.

In order to make certain that the tryptazan effect is not due to a differential toxicity of this compound

Table 1. Effect of Post-irradiation Incubation on the Yield of Prototrophs. $6{\cdot}1 \times 10^8$ Tryptophan-requiring *E. coli* per ml. were Irradiated with Ultra-violet to give a Survival of $4{\cdot}6 \times 10^7$ Cells/ml. (7 per cent). All Treatments were in Minimal Medium *C* supplemented as Indicated

	Post ultra-violet treatment	Viable cells plated	Mutants/plate	Prototrophs/10^8
	None	$4{\cdot}6 \times 10^6$	3·3	0·72
a	1 hr. AA, no tryptophan	$5{\cdot}2 \times 10^5$	1·0	2
b	As in (*a*), followed by an additional hour in AA plus tryptophan	$7{\cdot}7 \times 10^5$	97·6	127
c	1 hr. in AA plus tryptophan	$6{\cdot}3 \times 10^5$	126	200
d	As in (*c*), plus an additional hour in fresh medium of the same composition	$8{\cdot}2 \times 10^5$	144	175
e	1 hr. in AA plus methyl tryptophan	$3{\cdot}8 \times 10^5$	6	16
f	As in (*e*), but followed by an additional hour in AA plus tryptophan	$5{\cdot}9 \times 10^5$	96·6	164
g	1 hr. in AA plus tryptazan	$7{\cdot}0 \times 10^5$	0	0
h	As in (*g*), but followed by an additional hour in AA plus tryptophan	$5{\cdot}7 \times 10^5$	11	19

Table 2. Effect of 5-Methyl Tryptophan and of Tryptazan on Prototroph Viability. $3{\cdot}5 \times 10^9$ cells per ml. were Irradiated with Ultra-violet to give a Survival of $4{\cdot}6 \times 10^8$ Viable Cells per ml. (13 per cent)

	Post ultra-violet treatment	Viable cells plated	Mutants/plate	Prototrophs/10^8
	None	$4{\cdot}6 \times 10^7$	1·3	2·9
a	1 hr. in AA plus tryptophan	$3{\cdot}1 \times 10^7$	198	640
b	As in (*a*), followed by 1 hr. in AA plus tryptazan	$2{\cdot}5 \times 10^7$	188	750
c	As in (*a*), followed by 1 hr. in AA plus 5-methyl tryptophan	$3{\cdot}4 \times 10^7$	159	470
d	As in (*a*), followed by 1 hr. in AA plus tryptophan	$2{\cdot}2 \times 10^7$	227	1,003

on the prototrophs, the following experiment was performed (Table 2). Irradiated cultures were incubated for 1 hr. in medium containing an amino-acid mixture with tryptophan added, after which the cultures were washed, incubated in minimal medium with amino-acids plus tryptazan for 1 hr., rewashed and plated on minimal medium *C*. There is no inhibitory effect of tryptazan on cells which have already been fixed by preliminary incubation in amino-acids plus tryptophan.

It is apparent that there is a striking difference in the effect of the tryptophan analogues 5-methyl tryptophan and tryptazan. In the presence of methyl tryptophan the 'mutagen' is stable, with tryptazan it disappears. We feel that this difference is related to the different use made of these analogues by *E. coli*. Tryptazan is activated by the amino-acid activating enzyme[4] and is incorporated into the protein of *E. coli*[5]. Methyl tryptophan is neither activated nor incorporated and in fact inhibits the activating enzyme. However, the proteins formed by the incorporation of analogues are often inactive biologically[6].

We suppose that the 'mutagen' produced by ultra-violet irradiation is consumed in some way as a result of the protein synthesis necessary for mutation fixation. Formation of an abnormal protein, with tryptazan substituted for tryptophan, results in disappearance of the mutagen, but without the production of mutations for which active protein is necessary. The mutagen is stable when protein synthesis is not possible, as in the presence of 5-methyl tryptophan.

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