Chloramphenicol, nucleic acid synthesis and mutation induced by ultraviolet light

Several recent findings[1,2] suggest that DNA synthesis is the terminal event in u.v.-induced mutation in bacteria. Mutation expression follows closely the initial synthesis of DNA in the culture following u.v. exposure. Further, other studies[3–6] have shown that post-irradiation treatments blocking RNA or protein synthesis (*i.e.* incubation with 6-aza-uracil or chloramphenicol) are effective in lowering markedly the mutation-frequency response to ultraviolet light. Various investigators[7–9] have demonstrated that blockage of RNA or protein synthesis delays the recovery of DNA synthesis in u.v.-irradiated bacteria. It has recently been suggested by LIEB[10] that chloramphenicol reduces mutation frequency in bacteria by delaying DNA synthesis.

This question was investigated through studies of u.v.-induced reversion to prototrophy of the tryptophan-requiring auxotroph, *Escherichia coli* strain WP2. The techniques used have been previously described[5,6].

Table I demonstrates that when chloramphenicol is added to the incubating culture immediately after irradiation there is a reduction in the expected induced mutation frequency to a minimal level within 20 min. However, this treatment has no effect on subsequent DNA synthesis in the culture. Therefore, the contention[10] that chloramphenicol causes a lowered mutation-frequency response through delay of DNA synthesis does not appear plausible. It appears more reasonable that blockage of protein synthesis promotes an *active* "mutation-frequency decline" process as previously suggested by us[5,6].

TABLE I

EFFECT OF CHLORAMPHENICOL ON MUTATION FREQUENCY AND ON SUBSEQUENT DNA SYNTHESIS

*Time (min) with chloramphenicol**	*Prototrophs per 10^6 surviving auxotrophs (75 min)***	*Relative amount DNA with incubation (min)*			
		45	*60*	*75*	*90*
0	112	1.1	1.5	2.1	2.7
20	20	1.1	1.5	2.1	2.6

* After u.v. exposure the bacteria were suspended in growth medium containing 20 μg/ml chloramphenicol. After the indicated period of incubation the bacteria were rapidly centrifuged out of this media, resuspended in fresh media without chloramphenicol and incubation continued.

** Between 20 and 80 min the mutation frequency does not vary.

We have demonstrated[6] that the loss of sensitivity of potential mutations to the chloramphenicol promoted "mutation-frequency decline" process is correlated with initial doubling of RNA in the culture. We have designated[5] this loss of sensitivity "mutation fixation". Table II demonstrates that a relation exists between the amount of post-irradiation RNA synthesized at the time of chloramphenicol addition, the relative rate of DNA synthesis in the presence of chloramphenicol and the mutation-frequency response observed upon plating. Recovery of the capacity to synthesize DNA after u.v. exposure is related to the initial post-irradiation doubling of RNA in the culture. Apparently, the necessary RNA and protein must be formed prior

Abbreviations: RNA, ribonucleic acid; DNA, deoxyribonucleic acid; u.v., ultraviolet light.

to chloramphenicol addition in order for DNA to be replicated with consequent establishment of mutation in the genome. Where this mandatory RNA and protein synthesis has not occurred prior to chloramphenicol addition the potentiality for mutation is lost, presumably through the active decline process.

TABLE II

RELATION BETWEEN "MUTATION FIXATION", THE AMOUNT OF RNA SYNTHESIZED PRIOR TO CHLORAMPHENICOL ADDITION AND SUBSEQUENT RATE OF DNA SYNTHESIS IN THE PRESENCE OF CHLORAMPHENICOL

Time of chloramphenicol addition (min)*	*Mutation fixation*** %	*RNA*** increase in* %	*DNA synthesis****				
			relative amount with incubation (min)				*% of maximum rate in chloramphenicol*
			60	*70*	*80*	*90*	
0	16.7	0	1.06	1.02	1.08	1.00	0
30	22.2	17	1.12	1.19	1.24	1.33	20
40	32.9	36	1.18	1.24	1.43	1.53	40
50	61.3	63	1.18	1.38	1.58	1.77	62
60	93.6	102	1.23	1.55	1.87	2.06	97

* After u.v. exposure the cells were diluted into growth media and incubated. Chloramphenicol (20 μg/ml) was added at the indicated time. All times measured from start of incubation following u.v. exposure.

** Mutation frequency after incubation for 45 min in chloramphenicol (a period of time sufficient to eliminate all chloramphenicol-sensitive mutations) expressed as per cent of maximum frequency.

*** DNA synthesis in the presence of chloramphenicol. RNA formed prior to chloramphenicol addition.

Blockage of DNA synthesis during the "chloramphenicol challenge" period has no effect on the mutation frequency observed[11]. This finding is confirmed by use of 6-aza-uracil instead of chloramphenicol as the "mutation-challenging" agent. 6-aza-uracil blocks net RNA, protein and DNA synthesis but gives an identical "mutation-fixation" curve to that demonstrated by chloramphenicol[12]. Thus the hypothesis recently presented by LIEB[10] that the amount of DNA synthesis occurring in the presence of chloramphenicol in 60 min is significant in determining "mutation fixation" appears to be without validity. It is evident that the acquisition of the *capacity* to synthesize DNA rather than DNA synthesis is involved in "mutation fixation". Following u.v. exposure, the synthesis of RNA and protein endows the bacterium with the capacity both for DNA synthesis and mutation induction. The nature of the function of the RNA and protein in genetic replication and mutational change remains to be determined.

This work was supported in part by a grant from the U.S. National Institutes of Health and by U.S. Atomic Energy Commission Contract AT-(40-1)-2139.

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1 F. L. HAAS AND C. O. DOUDNEY, *Proc. Natl. Acad. Sci. U.S.*, 45 (1959) 1620.
2 F. L. HAAS AND C. O. DOUDNEY, *Nature*, 185 (1960) 637.
3 E. M. WITKIN, *Cold Spring Harbor Symposia Quant. Biol.*, 21 (1956) 123.
4 F. L. HAAS AND C. O. DOUDNEY, *Proc. Natl. Acad. Sci. U.S.*, 43 (1957) 871.

[5] C. O. DOUDNEY AND F. L. HAAS, *Proc. Natl. Acad. Sci. U.S.*, 44 (1958) 390.
[6] C. O. DOUDNEY AND F. L. HAAS, *Proc. Natl. Acad. Sci. U.S.*, 45 (1959) 709.
[7] F. M. HAROLD AND Z. Z. ZIPORIN, *Biochim. Biophys. Acta*, 29 (1958) 439.
[8] M. DRACULIC AND M. ERRERA, *Biochim. Biophys. Acta*, 31 (1959) 459.
[9] C. O. DOUDNEY, *Nature*, 184 (1959) 189.
[10] M. LIEB, *Biochim. Biophys. Acta*, 37 (1960) 155.
[11] C. O. DOUDNEY AND F. L. HAAS, unpublished observations.
[12] C. O. DOUDNEY AND F. L. HAAS, *Records Genetics Soc. America*, (1959) 66.

Received March 8th, 1960

6-Hydroxylation, the major metabolic pathway for melatonin

LERNER *et al.*[1–4] have demonstrated the presence of a melanocyte-contracting substance, melatonin (N-acetyl-5-methoxytryptamine), in bovine pineal glands and in the peripheral nerves of man, monkey, and cattle. AXELROD AND WEISSBACH[5] found a methyl transferase in pineal glands which can O-methylate N-acetylserotonin to melatonin. Little is known about the metabolic fate of this hormone in the body. Although LERNER *et al.*[6] have demonstrated 5-methoxyindole-acetic acid in bovine pineal glands, the origin of this compound is uncertain.

In order to study the metabolic fate of this hormone, [2-^{14}C]melatonin was synthesized from [2-^{14}C]serotonin, and administered intraperitoneally to albino male rats and the urine collected. Chromatography of the urine on Whatman No. 1 paper in isopropanol–5 % ammonia (8:2) and subsequent scanning for radioactive peaks indicated the presence of at least three radioactive compounds (R_F = 0.14, 0.54 and 0.75) the second peak (R_F = 0.54) containing about 80 % of the excreted radioactivity. Only two peaks (R_F = 0.25, 0.80) were found in butanol–acetic acid–water (4:1:1). None of the peaks had R_F's corresponding to melatonin, 5-methoxytryptamine, or 5-methoxyindoleacetic acid. A similar distribution of radioactivity was found on chromatography of the urine of rats which had received [^{14}C-*methoxy*]-melatonin or [^{3}H-*acetyl*]melatonin intraperitoneally indicating that the major metabolites retained both the 5-methoxy and the N-acetyl groups.

After the administration of a large dose of melatonin (20 mg), chromatography of the urine and spraying with Ehrlich's reagent indicated the presence of two indolic compounds, having the same R_F's as the first two radioactive metabolites in the isopropanol–ammonia system (R_F = 0.14, 0.54). These observations indicated that the α-position of the indole ring was unsubstituted. Hydrolysis of the eluted major component with a sulfatase preparation (Gluculase, Endo Products, N.Y.) in the presence of an antioxidant (ascorbic acid) or under N_2 resulted in the formation of a compound having a different R_F in butanol–acetic acid–water (4:1:1) [R_F = 0.67]. No change in R_F followed treatment with bacterial β-glucuronidase (Sigma Chemical Co., St. Louis). The hydrolysed compound reacted with diazotized *p*-nitroaniline to form a purple-colored derivative indicating the presence of a free phenolic group. Both hydrolysed and unhydrolysed metabolites gave red-colored compounds with acidic diazotized sulfanilic acid, the compound containing the free phenolic group reacting more rapidly. This reaction is characteristic of 6-hydroxyindoles and their sulfates[7,8]. Additional evidence for the identity of this metabolite as N-acetyl-5-