REFERENCES

1 R. C. WILLIAMS, *Advances Virus Research*, 2 (1954) 184.
2 T. F. ANDERSON, *Trans. N.Y. Acad. Sci.*, II, 13 (1951) 130.
3 R. C. WILLIAMS, *Exptl. Cell. Research*, 4 (1953) 188.
4 C. E. HALL, *J. Biophys. Biochem. Cytol.*, 1 (1955) 1.
5 H. E. HUXLEY, *First European Conference on Electron Microscopy, Stockholm, 1956*, p. 260.
6 S. BRENNER, G. STREISINGER, R. W. HORNE, S. P. CHAMPE, L. BARNETT AND S. BENZER, *J. Mol. Biol.*, 1 (1959) in the press.
7 D. E. BRADLEY, *Brit. J. Appl. Phys.*, 5 (1954) 65.
8 C. E. HALL AND M. LITT, *J. Biophys. Biochem. Cytol.*, 4 (1958) 1.
9 V. E. COSSLETT AND R. W. HORNE, *Vacuum*, 5 (1955) 109.
10 R. FRANKLIN AND K. C. HOLMES, *Acta Cryst.*, 11 (1958) 213.
11 R. FRANKLIN AND A. KLUG, *Biochim. Biophys. Acta*, 19 (1956) 403.
12 R. HART, *Biochim. Biophys. Acta*, 28 (1958) 457.
13 H. FRAENKEL-CONRAT, *Virology*, 4 (1957) 1.
14 A. KLUG, J. T. FINCH AND R. FRANKLIN, *Biochim. Biophys. Acta*, 25 (1957) 242.
15 R. L. STEERE, *J. Biophys. Biochem. Cytol.*, 3 (1957) 45.
16 R. MARKHAM, *Discussions Faraday Soc.*, 11 (1951) 221.

ALTERATIONS IN THE RADIOSENSITIVITY OF *ESCHERICHIA COLI* THROUGH MODIFICATION OF CELLULAR MACROMOLECULAR COMPONENTS

DANIEL BILLEN

M. D. Anderson Hospital and Tumor Institute, University of Texas, Houston, Texas (U.S.A.)

(Received October 6th, 1958)

SUMMARY

The number of survivors of *E. coli* strains B/r or 15_T^- following X-ray exposure was increased if the log phase cells had been previously grown in the presence of chloramphenicol. By omission of thymine in the growth medium of *E. coli* strain 15_T^- the chloramphenicol effect failed to materialize. Under these conditions net DNA synthesis was prevented while RNA continued to be accumulated. Since protein synthesis was inhibited by chloramphenicol under all conditions employed it would appear that the "surplus" DNA was responsible for the enhanced survival of cells so treated.

INTRODUCTION

While numerous investigations have been made on the relative roles of the nuclear and cytoplasmic nucleic acids in radiosensitivity of animal and plant cells, similar information on bacterial cells has been difficult to obtain because of the refractory nature of such cells to the usual physical manipulations found successful with the larger cells. Recently, however, several means of altering the ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and protein (P) contents of bacteria have been described[1,2]. In the present work alterations in macromolecular components have been

References p. 116.

induced in an attempt to determine the relative roles of these components in bacterial cell radiosensitivity.

METHODS

Organisms and conditions of growth

Escherichia coli strain B/r was grown with aeration at 37° for 16 h in a minimal-salts medium of the following composition per liter distilled water: 1 g KH_2PO_4, 0.7 g $MgSO_4 \cdot 7H_2O$, 1 g NaCl, 4 g $(NH_4)_2HPO_4$, 0.5 g sodium citrate, 0.1 ml of a 0.5% solution of $FeCl_3$, and 10 g of glucose sterilized separately. The final pH was 6.8 following the necessary adjustments with HCl and NaOH. The thymine requiring *E. coli* strain 15_{T^-} was similarly grown with the addition of thymine (20 μg/ml final concentration) to the minimal medium. For every 100 ml of fresh medium a 2.5 ml inoculum of the 16 h cultures was used. This provided an initial population of $1-4 \cdot 10^7$ cells/ml. Such a culture upon re-incubation with aeration at 37° reached log phase by 2 h. This was determined by turbidity measurements as well as platings. All platings were done on the minimal medium supplemented with Difco Noble Agar unless otherwise indicated. When the population reached an optical density of approximately 0.50 at 420 mμ as measured by a Bausch and Lomb, Spectronic 20 Colorimeter using a tube of 16.8 mm I.D., a sample of the culture was withdrawn for the determination of the number of viable cells as well as protein nitrogen (N_P), RNA, and DNA. The number of viable cells at this time was of the order of $2-5 \cdot 10^8$ cells/ml. To the remaining cultures were added the several chemicals desired and the incubation continued. These cultures were terminated at the end of an hour. In the experiments concerned with the influence of thymine starvation on *E. coli* 15_{T^-} the cells were harvested and washed before addition of the several compounds. Upon completion of growth all cultures were chilled in an ice bath and the cells harvested in a refrigerated centrifuge. The cells were washed once in minimal medium and re-suspended in 1/10 the original volume of fresh minimal. For radiosensitivity studies a 1-ml aliquot was removed and diluted twenty-fold. Aliquots of this diluted suspension were exposed to the several doses of X-rays described or served as controls. The remaining concentrated cell suspensions were used for the chemical analysis.

RNA, DNA, and N_P determination

The 10 × concentrated washed cell suspension was made to 10% TCA at ice bath temperature. Following one hour's incubation in the refrigerator the precipitate was centrifuged out and the nucleic acids extracted by hydrolysis in 5% TCA at 100° for 30 min[3]. The remaining precipitate was washed once with 5% TCA and the supernatants pooled. Aliquots of the supernatant were tested for RNA by the orcinol method and for DNA with diphenylamine. Yeast nucleic acid served as the standard RNA preparation and polymerized salmon sperm DNA was used as the DNA standard. Both were commercial preparations obtained from California Foundation for Biochemical Research. The remaining precipitate was taken to be protein and its nitrogen content analyzed by the micro-kjeldahl method.

Irradiation procedure

Suspensions of *E. coli* were exposed to X-rays as follows: 1 ml of cell suspension

References p. 116.

was added to a 2-ml pyrex glass tube and a cork stopper applied. The tube was then inserted into a plastic container packed in ice as previously described[4]. The radiation factors for the Westinghouse machine used were as follows: 200 KVP; 15 mA constant potential; 1 mm of aluminum added filtration; h v l 0.8 mm Cu. The dose rate in air was 1100 R/min.

RESULTS

The influence of nucleic acid accumulation relative to protein content on radiosensitivity of E. coli *B/r*

When chloramphenicol (10 μg/ml final concentration) is added to a log phase culture of *E. coli* B/r synthesis of both RNA and DNA continues while the formation of protein is inhibited[1,2]. In our experiments incubation was limited to 1 h. The results given are calculated on a per cell basis since some division occurred during the hour's growth in the presence of chloramphenicol. This cell division was more pronounced for the thymineless strain as will be discussed later on. During this interval the RNA content of *E. coli* B/r was approximately doubled while the DNA content increased over 50 % (Table I). There was also some increase in N_P. In most experiments, however, the RNA and DNA to protein ratios were increased considerably following incubation of the cells in the chloramphenicol (Table I). When such cells were harvested and washed free of exogenous chloramphenicol they showed a greater number of colony-forming survivors following X-ray exposure than did the control cells (Fig. 1).

TABLE I

NUCLEIC ACID AND PROTEIN CONTENT OF *E. coli* B/r FOLLOWING CHLORAMPHENICOL TREATMENT FOR ONE HOUR

Experiment	Percent of increase over initial value (per cell)			Ratios					
				RNA/DNA		RNA/N_P		DNA/N_P	
	N_P	RNA	DNA	Initial	Final	Initial	Final	Initial	Final
1043	13	197	78	8.4	14.0	2.9	7.6	0.35	0.54
1049	4	112	55	9.6	13.1	3.23	7.05	0.35	0.54
1057	8	91	68	8.9	9.8	3.85	5.8	0.43	0.67

To determine if chloramphenicol could exert its effect without initiating "unbalanced" growth, stationary phase cells incubated for an hour in the presence of chloramphenicol were also exposed to X-rays. During this incubation period in the presence of chloramphenicol, stationary phase cells showed little growth and maintained normal RNA, DNA, and N_P values. The radiosensitivity of chloramphenicol pre-treated, stationary phase cells was equivalent to that of the control group. Plating on nutrient agar with or without yeast extract (1 %) supplement did not abolish the chloramphenicol effect on log phase cells relative to that of the controls, although a nutrient effect on survival was observed[4-6].

The influence of DNA synthesis on the radiosensitivity of E. coli 15_T^-

In order to facilitate manipulation of the nucleic acid content of the bacterial

References p. 116.

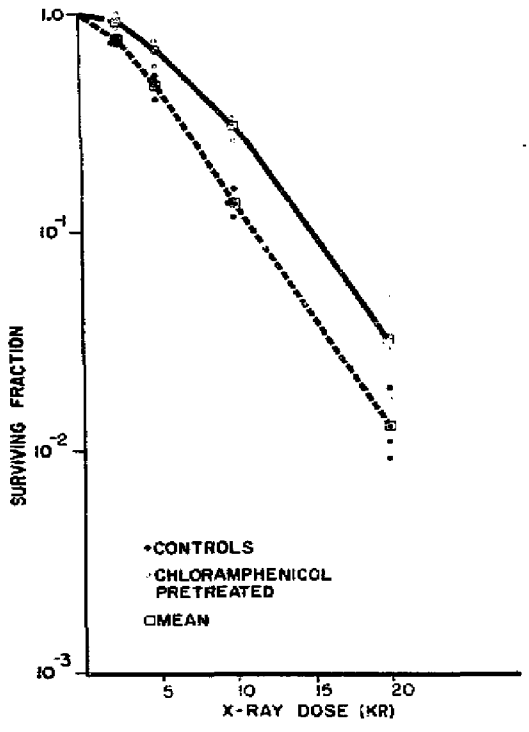

Fig. 1. Effect of pre-incubation in chloramphenicol on radiosensitivity of *E. coli* B/r. Incubation and subsequent treatment as described in text.

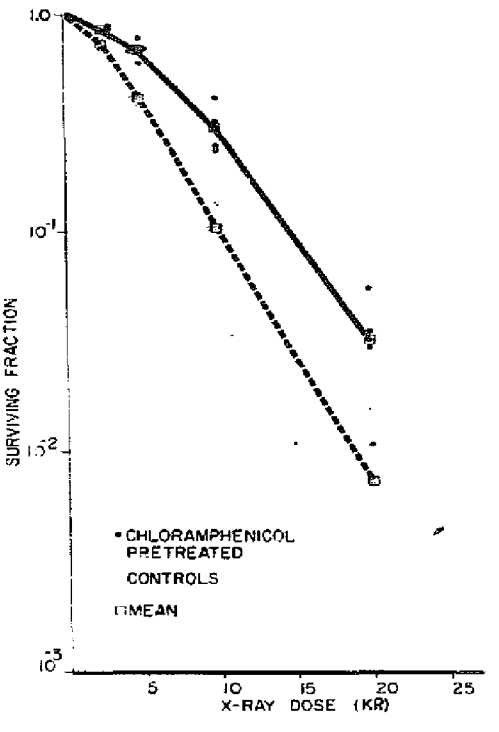

Fig. 2. Effect of pre-incubation in chloramphenicol on radiosensitivity of *E. coli* 15_{T-}. Incubation and subsequent treatment as described in text.

cells, the thymine-requiring *E. coli* 15_{T-} mutant isolated by BARNER AND COHEN[7] was also studied. When such cells were suspended in minimal medium without thymine, DNA synthesis was arrested, and a loss of colony-forming cells ensued. (Table II). Chloramphenicol was found to antagonize "thymineless" death (Table II). In Fig. 2 are presented survival data showing increased survivors when X-ray exposure followed incubation of growing log phase cells in a minimal medium containing thymine (20 μg/ml final concentration) plus chloramphenicol.

TABLE II

CHANGES IN NUMBER OF COLONY-FORMING *E. coli* 15_{T-} CELLS FOLLOWING A ONE-HOUR INCUBATION WITH AND WITHOUT THYMINE AND/OR CHLORAMPHENICOL

Medium composition	*Initial (× 10^{-8}) Experiment*		*Final (× 10^{-8}) Experiment*		*Percent of change Experiment*	
	1069	*1072*	*1069*	*1072*	*1069*	*1072*
Minimal plus thymine	2.29	2.02	4.50	3.90	+ 96	+ 93
Minimal plus thymine plus chloramphenicol	2.29	2.03	3.00	2.48	+ 31	+ 23
Minimal only	2.29	2.02	0.73	1.22	— 68	— 40
Minimal plus chloramphenicol	2.29	2.02	1.89	1.95	— 17	— 4

References p. 116.

An analysis of the relative protein, RNA and DNA content of such cells following incubation for 1 h under the conditions described is given in Table III. On a per ml basis the increment in RNA and DNA following 1 h of incubation in complete medium plus chloramphenicol approached that found with B/r. However, when calculated on a per cell basis the increase in RNA and DNA was not as pronounced and a decreased protein content per colony former was observed. This may be accounted for by the surprisingly large number of cells dividing in the presence of chloramphenicol (Table II).

In the absence of thymine, RNA synthesis continued in all cells independent of the presence or absence of chloramphenicol while DNA synthesis was inhibited (Table IV). The RNA content was thus greatly increased relative to that of the DNA

TABLE III

NUCLEIC ACID AND PROTEIN CONTENT OF *E. coli* STRAIN 15T⁻ FOLLOWING CHLORAMPHENICOL TREATMENT FOR ONE HOUR

Experiment	Percent change over initial value			Ratios					
				RNA/DNA		RNA/N_P		DNA/N_P	
	N_P*	RNA*	DNA*	Initial	Final	Initial	Final	Initial	Final
1071	— 30 (+ 24)**	+ 10 (+ 96)	+ 7 (+ 91)	8.1	8.2	5.2	8.35	0.65	1.00
1074	— 25 (+ 11)	+ 54 (+ 127)	+ 34 (+ 98)	7.7	8.8	3.4	7.0	0.45	0.80
1063	— 28 (+ 13)	+ 22 (+ 91)	+ 33 (+ 95)	8.5	8.4	4.15	7.0	0.49	0.85

* Calculated per viable cell.
** Figures in parenthesis calculated on a per ml basis.

TABLE IV

ALTERATION IN NUCLEIC ACID AND PROTEIN CONTENT OF *E. coli* STRAIN 15T⁻

Medium composition	μg cell⁻¹ (×10⁸)		
	N_P	RNA	DNA
Minimal plus thymine	3.46	12.00	1.58
Minimal plus thymine and chloramphenicol	2.65	20.80	2.10
Minimal only*	6.95	25.40	1.45
Minimal plus chloramphenicol*	3.32	25.80	1.53

* Initial number of viable cells used in calculations since thymineless death occurs under this condition.

TABLE V

COMPARISON OF EFFECTS OF PRE-TREATMENT ON THE RADIATION SENSITIVITY OF *E. coli* STRAIN 15T⁻

Medium composition	Surviving fraction	
	10 kR	20 kR
Minimal plus thymine	$3.3 \cdot 10^{-2}$	$1.29 \cdot 10^{-3}$
Minimal plus thymine and chloramphenicol	$3.2 \cdot 10^{-1}$	$1.1 \cdot 10^{-2}$
Minimal only	$6.35 \cdot 10^{-3}$	$6.15 \cdot 10^{-4}$
Minimal plus chloramphenicol	$1.12 \cdot 10^{-2}$	$4.55 \cdot 10^{-4}$

References p. 116.

and protein when the cells were incubated in a medium lacking thymine, but containing chloramphenicol. Protein synthesis continued unabated for this hour period in the absence of chloramphenicol. Cells maintained in the absence of thymine were found to be more radiosensitive than thymine-grown cells (Table V). Contrary to the results observed for thymine-grown cells the addition of chloramphenicol did not alter the radiosensitivity of such cells (Table V). As summarized in Table VI, analysis of the radiosensitivity of the cells following the several treatments showed that growth in the presence of chloramphenicol induced radioresistance. This resistance was lost when the cells were unable to synthesize DNA in the thymineless medium. Alterations in the protein and RNA constituents of the cells were apparently unrelated to the induction of radioresistance.

TABLE VI

RELATIONSHIP BETWEEN ALTERATION IN P, RNA, DNA CONTENT, AND X-RAY-INDUCED DEATH OF *E. coli* 15_{T-}

Pretreatment	*Change in cell content of*			*Effect on number of survivors*
	P	*RNA*	*DNA*	
1 (Thymine addition)	o	o	o	o
2 (Chloramphenicol and thymine addition)	—	+	+	+
3 (Thymine omission)	+	+	o	—
4 (Condition 3 plus chloramphenicol addition)	o	+	o	—

o Little or no change.
+ Increased content or survivors.
— Decreased content or survivors.

DISCUSSION

The data suggest that altering the DNA content of a cell influences its response to X-ray exposure. When DNA synthesis was blocked by witholding thymine from *E. coli* 15_{T-} the chloramphenicol-induced radioresistance did not materialize. Non-treated cells grown in thymine-deficient medium were found to be more radiosensitive than cells grown in the presence of thymine.

The implication of an increased cellular DNA content being responsible for the enhanced resistance following chloramphenicol pre-treatment may be a fortutious one. Metabolic changes, other than those reported here and in the literature, for the several macromolecular components studied, probably take place. Indeed ALLEN AND POWELSON report such changes in glucose metabolism although higher concentrations of chloramphenicol were employed in their study[8]. The possibility that the induced division delay of chloramphenicol pre-treated cells[9] may contribute to the response cannot be overlooked. "Recovery" or "restoration" could occur during the increased lag phase. Investigations have been described which showed that sub-optimal growth conditions, *i.e.* low temperature[4], or the presence of inhibitors[6], following X-irradiation of *E. coli* enhanced the number of survivors. The absence of a chloramphenicol effect on radiosensitivity of stationary phase cells would suggest that antibiotic carry over is probably not involved. The observation that cells "unbalanced" only in their RNA content do not show the chloramphenicol effect, would

References p. 116.

imply a more specific role for the surplus DNA if in fact a post-irradiation "restoration" were occurring.

A sufficient number of reports concerning other attempts to modify sensitivity experimentally have been recorded to establish that conditions and stages of growth prior to exposure influence the radiation response of cells. In a number of these investigations the increased resistance would appear to be the result of either increased ploidy[10,11] or nuclear number [12-14]. In either case a related increase in protein, RNA and DNA would be expected. MORSE AND CARTER[3] found that the radioresistant *E. coli* strain B/r contained three to four times as much DNA as its sensitive parent, strain B. HOLLAENDER *et al.*, showed that *E. coli* B/r grown anaerobically were more resistant to X-rays than aerobically grown cells[15]. In light of the findings of COHEN[16] that anaerobic fermentation of glucose favors deoxyribose formation it would be of interest to determine the relative DNA content of such cells. Enhanced resistance to ultraviolet light has been reported in *E. coli* cells with increased DNA content brought about by growth either in the presence of copper[17] or the presence of the amino acid analog B-2-thienylalanine[18]. MCFALL and collaborators[19], as a result of their studies on ^{32}P decay in bacterial DNA and RNA, concluded that it was the disintegrations occurring in the DNA that was primarily responsible for the decay-induced loss of viability in *E. coli*. The results of our studies would lead to a similar conclusion with regard to the involvement of DNA in radiation death.

ACKNOWLEDGEMENTS

The author would like to thank Mr. W. MCAFEE and Mrs. G. WILLARD for their technical assistance during this investigation. The use of the facilities and services of the Physics Department of this Institution is hereby gratefully acknowledged.

REFERENCES

1 C. L. WISSEMAN, JR., J. E. SMADEL, F. E. HAHN AND H. E. HOPPS, *J. Bacteriol.*, 67 (1954) 662.
2 E. F. GALE AND J. P. FOLKES, *Biochem. J.*, 45 (1953) 493.
3 M. L. MORSE AND C. E. CARTER, *J. Bacteriol.*, 58 (1949) 317.
4 G. E. STAPLETON, D. BILLEN AND A. HOLLAENDER, *J. Cellular Comp. Physiol.*, 41 (1953) 345.
5 G. E. STAPLETON, A. J. SBARRA AND A. HOLLAENDER, *J. Bacteriol.*, 70 (1955) 7.
6 T. ALPER AND N. E. GILLIES, *J. Gen. Microbiol.*, 18 (1958) 461.
7 H. D. BARNER AND S. S. COHEN, *J. Bacteriol.*, 68 (1954) 80.
8 G. S. H. ALLEN, JR. AND D. M. POWELSON, *Science*, 127 (1958) 1341.
9 F. E. HAHN, M. SCHAECHTER, W. S. CEGLOWSKI, H. E. HOPPS AND J. CIAK, *Biochim. Biophys. Acta*, 26 (1957) 467.
10 R. LATERJET AND B. EPHRUSSI, *Compt. rend.*, 229 (1949) 306.
11 A. M. CLARK AND E. M. KELLY, *Cancer Research*, 10 (1950) 348.
12 G. E. STAPLETON, *J. Bacteriol.*, 70 (1955) 357.
13 K. C. ATWOOD, *Genetics*, 37 (1952) 564.
14 M. R. ZELLE AND A. HOLLAENDER, in A. HOLLAENDER, *Radiation Biology*, Vol. II, McGraw Hill Book Co., Inc., 1955, p. 365.
15 A. HOLLAENDER, G. E. STAPLETON AND F. L. MARTIN, *Nature*, 167 (1951) 103.
16 S. S. COHEN, *Nature*, 168 (1951) 746.
17 L. L. WEED AND D. LONGFELLOW, *J. Bacteriol.*, 67 (1954) 27.
18 A. B. PARDEE AND L. S. PRESTIDGE, *Biochim. Biophys. Acta*, 27 (1958) 412.
19 E. MCFALL, A. B. PARDEE AND G. S. STENT, *Biochim. Biophys. Acta*, 27 (1958) 282.