[21] B. MALMGREN AND C. HEDEN, *Acta Path. Microbiol. Scand.*, 24 (1947) 472.
[22] T. CASPERSSON, *Soc. Exptl. Biol. Symposia*, 1 (1947) 127.
[23] J. BRACHET, *Cold Spring Harbor Symposia Quant. Biol.*, 12 (1947) 18.
[24] M. R. MCDONALD ,in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. II, Academic Press, 1955, p. 427.
[25] S. J. SINGER, personal communication.
[26] C. ANFINSEN, *The Molecular Basis of Evolution*, J. Wiley and Sons, 1959, p. 99.
[27] D. ELSON, *Biochim. Biophys. Acta*, 27 (1958) 207; 216.

Biochim. Biophys. Acta, 43 (1960) 173–182

THE NUCLEIC ACID CONTENT OF *ESCHERICHIA COLI* STRAINS B AND B/R

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SUMMARY

The differences in the DNA and RNA content of cells of *Escherichia coli* strain B and its radio-resistant mutant, strain B/r, in the logarithmic phase of growth are not statistically significant. In the stationary phase, cells of strain B/r contain more DNA than those of strain B, but the difference is barely significant; the RNA content of the B/r cells is significantly higher. The differing radiosensitivities of the two strains cannot be correlated with differences in their content of nucleic acids.

INTRODUCTION

In recent years considerable emphasis has been placed on the possible connection between DNA and the specific site of damage in living cells exposed to ionizing or u.v. radiations. It is sometimes assumed that the lethal effect of radiation (*i.e.* inhibition of clone formation) is primarily due to damage to the DNA itself. Direct correlation between the DNA content of cells and their radiosensitivity might support this assumption, although lack of correlation is, of course, not evidence against it. Several authors have attempted to relate radiosensitivity to DNA content. Thus PARDEE AND PRESTIDGE[1] concluded that a relative surplus of DNA over protein and RNA in cells of *E. coli* B was probably responsible for reducing the u.v. sensitivity of bacteria which had been treated with β-2-thienylalanine. BILLEN[2] implied that the reduction of X-ray sensitivity, conferred by incubating *E. coli* B/r and *E. coli* 15T$^-$ with chloramphenicol before irradiation, was due to an increase in the DNA/protein ratio of the cells. These papers exemplify the line of reasoning that the radio-

Abbreviations: DNA, deoxyribonucleic acid; RNA, ribonucleic acid.

sensitivity of cells of the same strain can be negatively correlated with their DNA content, and that if a radio-resistant mutant develops from a parent strain, this can be attributed to the greater DNA content of the mutant. On the other hand, it is possible to argue, as PUCK[3] has done, that the more DNA there is present in a cell the greater will be the target presented to the damaging radiation, and therefore radiosensitivity will increase with an increase of DNA content.

A well known example of a radio-resistant mutant is the strain B/r isolated by WITKIN[4] from *E. coli* strain B, and the report by MORSE AND CARTER[5], that *E. coli* B/r cells contain more DNA than those of *E. coli* B, has frequently been quoted. The relevance of the nucleic acid content of *E. coli* B and its mutant to their differing radiosensitivities became very questionable when HAROLD AND ZIPORIN[6] were unable to confirm the results of MORSE AND CARTER[5], and reported in contrast that the concentration of DNA in the two strains was the same. These conflicting results have led us to examine the nucleic acid contents of the two strains, in different physiological stages, paying particular attention to the errors which arise in the measurements.

METHODS

Bacteria

Escherichia coli strain B and *Escherichia coli* strain B/r were maintained on slopes of Oxoid Blood Agar Base.

Growth of cells

A loopful from the stock slope was inoculated into 5 ml of Oxoid nutrient broth, which was then incubated, without aeration, for 18 h at 37°. This culture, containing approximately $5 \cdot 10^8$ cells/ml, was used to prepare batches of about 10^{10} logarithmic or stationary-phase cells.

Logarithmic-phase cells: A sample of the stock broth culture, containing about $5 \cdot 10^7$ cells, was added to each of 8 × 100 ml screw-cap bottles half filled with Oxoid nutrient broth. The bottles were incubated for 2.5 h under the aeration provided by incubating at 37° on a turntable rotating at an angle (Matburn Ltd., London). The cultures were cooled in melting ice, and the cells were centrifuged at room temperature, and resuspended in 20 ml of 0.067 *M*, pH-7 phosphate buffer.

Stationary-phase cells: Two bottles, inoculated from the stock broth culture, were incubated, with aeration, for 24 h at 37°, and the cells harvested as above.

Estimations of errors in analysis

In the chemical estimation of the concentration of any cellular constituent, errors are involved in both the counting of the cells and the chemical estimation.

Counting of cells: Both total and viable cell counts were made. For total counting, three unequal dilutions of the concentrated cell suspension were made in phosphate buffer, containing 1% formalin to prevent further cell division. These were coded so that the relative cell concentrations were unknown to the observer, who used a calibrated Helber bacterial counting chamber (Hawksley & Sons Ltd., London) to count the cells. The weighted mean of the three counts was used in estimating the nucleic acid content per cell, and the average errors in the counts were estimated from the error in each set of three counts.

DNA and RNA estimations: The concentrated suspension of cells was divided

into three unequal parts, which were subsequently treated alike. The means and the standard errors of the nucleic acid estimations were calculated from the three values obtained for each batch. The cells were removed from the buffer by centrifugation and the nucleic acids extracted with perchloric acid according to the method of BURTON[7]. The concentration of DNA in the acid extract was determined by its reaction with indole and HCl, by the method of DISCHE[8] as modified by CERIOTTI[9]. RNA was estimated with orcinol according to the modification of the method of BARRENSCHEEN AND PEHAM[10] introduced by CERIOTTI[11]. Reference standards were prepared from samples of DNA and RNA purchased from the Nutritional Biochemicals Corporation.

RESULTS

The standard error in chemical estimation of both DNA and RNA was within 4% of each mean estimate, assuming that a normal error curve applied to the three values obtained for each batch.

From 51 individual counts made in the series of experiments, it was determined that the standard error in making a count of n cells was $\sqrt{2n}$. Since a total of about 1200 cells was counted from each batch used in the chemical determinations, the average value of 4% was adopted as the standard error of counting. The importance of basing estimates of any cellular constituent on total and not on viable count is demonstrated in Table I.

TABLE I

THE NUCLEIC ACID CONTENT PER CELL OF *E. coli* STRAIN B AND *E. coli* STRAIN B/r IN TWO DIFFERENT STAGES OF THE GROWTH CYCLE

Strain	*Time of incubation (hr)*	% *viable cells*	*DNA* ($\times 10^{-8}$ µg/cell)	*RNA* ($\times 10^{-8}$ µg/cell)	*RNA/DNA*
E. coli B	2.5	93	1.53	8.66	5.68
		48	1.12	6.24	5.59
		72	1.46	8.63	5.91
Mean for group			1.37 ± 0.13	7.84 ± 0.79	5.73 ± 0.10
E. coli B/r	2.5	60	1.31	7.00	5.46
		69	1.78	12.60	7.12
		74	3.03	13.80	4.58
		—	1.38	8.79	6.39
		71	1.60	8.22	5.18
Mean for group			1.82 ± 0.30	10.08 ± 1.32	5.75 ± 0.45
E. coli B	24	16	0.70	0.65	0.92
		11	0.71	0.68	0.93
		12	0.92	0.62	0.68
Mean for group			0.78 ± 0.07	0.65 ± 0.02	0.84 ± 0.09
E. coli B/r	24	42	1.16	1.96	1.69
		45	1.13	2.53	2.23
		18	1.54	1.54	1.00
Mean for group			1.28 ± 0.13	2.01 ± 0.30	1.64 ± 0.36

The concentrations of DNA and RNA found per cell of *E. coli* B and *E. coli* B/r in different stages of growth are shown in Table I. The value given for the content

of DNA and RNA per cell in each batch is the mean of three determinations. The errors involved in counting the cells and in the chemical estimations are both included in the standard error attached to the mean value, which standard error is based on the variance of the three or more values whose mean has been taken. The results within each group show that the dispersion of the values between batches was greater than could be accounted for by combining the estimated errors in chemical procedures and in counting the cells. Although all batches of cells within each group were grown and harvested as far as possible in the same way, it is clear that uncontrolled variations arose from batch to batch. The largest number of batches examined in any one group was five (for logarithmic-phase cells of B/r), which is not large enough to determine the type of distribution of the values obtained. However, the values within this group are consistent with the assumption that there is a normal distribution of errors, and therefore the standard error of the mean value for each group has been calculated on this basis.

Comparisons were made between the group means for the DNA and RNA contents of the two strains both in the logarithmic and the stationary stages of growth, and also between the DNA and RNA contents of cells of the same strain in the two physiological stages. An analysis of variance was used to test the significance of differences between pairs of means. These comparisons show that (a) the differences in the DNA and the RNA content of the two strains in the logarithmic phase were not significant at $P = 0.05$ level; (b) the concentration of DNA in stationary-phase cells of *E. coli* B/r was just significantly higher than that in *E. coli* B ($P = 0.05$); (c) there was significantly more RNA in stationary-phase cells of strain B/r than in strain B (significant at $P = 0.05$ level and almost significant at $P = 0.01$); (d) the difference between the DNA contained in logarithmic-phase and stationary-phase cells of strain B was just significant ($P = 0.05$); (e) there was no significant difference in the DNA content of B/r cells in the different physiological states.

The ratios of RNA/DNA, which are rather more accurate as they are independent of counting errors, were the same for logarithmic-phase cells of the two strains, but this ratio was significantly higher for resting cells of strain B/r than for those of strain B. For both strains the absolute quantity of RNA, and the RNA/DNA ratio were very much greater in logarithmic than in stationary-phase cells. This increase in the RNA concentration in actively dividing bacterial cells has been reported by many authors (*e.g.* 12–14).

DISCUSSION

Few values for the nucleic acid contents of *E. coli* B and *E. coli* B/r are available in the literature. The only study comparable to our own was made by HAROLD AND ZIPORIN[6] on cells grown in a synthetic medium, and their results are shown in Table II. Their figures for DNA and RNA are very similar to our results for logarithmic-phase cells, but their values for these in stationary-phase cells are much higher throughout. The differences in the latter are probably due to the fact that HAROLD AND ZIPORIN[6] based the concentrations per cell on the viable and not on the total cell count of their cultures. Our results show that the viable cell count is frequently much lower than the total cell count, especially in populations of non-dividing cells. However, in general, the results of HAROLD AND ZIPORIN[6] are confirmed by our findings, that

there is no significant difference in the concentration of DNA in cells of the two strains in the same stages of growth. Figures kindly made available to us by HILL[15] again show (Table II) that the DNA and RNA contents of stationary-phase cells of strain B and strain B/r grown in nutrient broth are not appreciably different, although she found a higher content of RNA in *E. coli* B and a lower content of DNA in strain B/r than we have estimated.

MORSE AND CARTER[5] stated that cells of strain B/r contained 3–4 times the concentration of DNA in strain B, but they did not provide data in support of this, and the values which they quoted, for resting-stage cells grown in nutrient broth, do not show such a large difference (Table II). It is surprising that they found so much more DNA than RNA in stationary-phase cells of both strains.

TABLE II

COMPARISON OF THE RESULTS OBTAINED BY DIFFERENT WORKERS FOR THE NUCLEIC ACID CONTENT OF *E. coli* STRAIN B AND *E. coli* STRAIN B/r

Values are expressed as 10^{-8} μg of DNA or of RNA per cell.

			Present work	HAROLD AND ZIPORIN *(1958)*	HILL *(1960)*	MORSE AND CARTER *(1949)*
Logarithmic-phase cells	*E. coli* B	DNA	1.37	1.6–1.8	—	—
		RNA	7.84	10.0–12.0	—	—
	E. coli B/r	DNA	1.82	1.6–1.8	—	—
		RNA	10.08	10.0–12.0	—	—
Stationary-phase cells	*E. coli* B	DNA	0.78	2.4–2.6	0.75	1.4, 1.6
		RNA	0.65	6.5–7.5	4.20	0.9, 0.5
	E. coli B/r	DNA	1.28	2.2–2.7	0.62	3.5
		RNA	2.01	5.0–6.5	2.90	1.8

McFall (quoted by PARDEE AND PRESTIDGE[1]) calculated a DNA/RNA ratio for *E. coli* B/r which was twice that for strain B. No indication of the metabolic state of these cells was given, but this ratio, calculated from our data, is the same for logarithmic-phase cells of the two strains, and its value for resting cells of strain B/r is half that for similar cells of strain B.

Our results show that there is no significant difference in the concentration of DNA in logarithmic-phase cells of strains B and B/r grown in nutrient broth, while the difference in the amount of DNA contained in the resting cells of the two strains is small. It is just "significant" at the $P = 0.05$ level but doubt is thrown on the significance of this result by our lack of knowledge of the true distribution of values within a group, nor is it known whether the two strains were equally variable from batch to batch. The lethal effect of radiations on cells of these strains is markedly influenced by conditions after irradiation[16–19], but in general considerably higher doses of radiation are required to bring about the same lethal effect in strain B/r as in *E. coli* B. It is difficult to assign a numerical value to the ratios of radiosensitivities, as the shapes of the survival curves differ, but if we use as an arbitrary standard the doses required to reduce each surviving population to 10%, then the ratio of sensitivities may vary from one, for logarithmic-phase cells incubated on

minimal medium at 37° after exposure to u.v. light[19], to 25, for resting cells irradiated with X-rays under oxygen and incubated at 19° on a nutrient medium[20]. Non-dividing cells of strain B are more resistant to u.v. light and X-rays than are dividing cells[18], but they contain perhaps slightly less, and certainly no more DNA. The radiosensitivities of cells of strain B/r differ more widely between these two physiological states, but the difference lies essentially in the point to which the exponential part of the survival curve extrapolates in a semi-logarithmic plot. The intercept which the extrapolated curve makes with the ordinate is usually called the "multiplicity", but we propose to refer to it as the "extrapolation number"[21]. A variation in extrapolation number, but not in the slope, may be remarked in the survival curves found by HOLLAENDER *et al.*[22] for cells of strain B/r grown in different ways. Similarly, the difference in sensitivity noted by BILLEN[2] for cells of B/r incubated before irradiation in a medium containing chloramphenicol is due to a difference in the extrapolation number. The extrapolation number for u.v.-irradiated *E. coli* strain B/r incubated on a minimal medium has been found to vary from one, for logarithmic-phase cells, to about 3 for stationary-phase cells, but there is no significant difference in the DNA contents of the cells in the two stages.

We conclude from this analysis that the radiosensitivity of strains B and B/r is not correlated, positively or negatively, with the DNA or RNA contents of cells of these strains.

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REFERENCES

1 A. B. PARDEE AND L. S. PRESTIDGE, *Biochim. Biophys. Acta*, 27 (1958) 412.
2 D. BILLEN, *Biochim. Biophys. Acta*, 34 (1959) 110.
3 T. T. PUCK, *Rev. mod. Phys.*, 31 (1959) 433.
4 E. M. WITKIN, *Genetics*, 32 (1947) 221.
5 M. L. MORSE AND C. E. CARTER, *J. Bacteriol.*, 58 (1949) 317.
6 F. M. HAROLD AND Z. Z. ZIPORIN, *Biochim. Biophys. Acta*, 28 (1958) 482.
7 K. BURTON, *Biochem. J.*, 62 (1956) 315.
8 Z. DISCHE, *Biochem. Z.*, 204 (1929) 431.
9 A. CERIOTTI, *J. Biol. Chem.*, 198 (1952) 297.
10 H. BARRENSCHEEN AND A. PEHAM, *Z. physiol. Chem., Hoppe Seyler's*, 272 (1942) 81.
11 A. CERIOTTI, *J. Biol. Chem.*, 214 (1955) 59.
12 P. C. CALDWELL, E. L. MACKOR AND C. HINSHELWOOD, *J. Chem. Soc.*, (1950) 3151.
13 M. SCHAECHTER, O. MAALØE AND N. O. KJELDGAARD, *J. Gen. Microbiol.*, 19 (1958) 592.
14 J. MENDELSOHN AND A. TISSIÈRES, *Biochim. Biophys. Acta*, 35 (1959) 248.
15 R. F. HILL, in preparation.
16 R. B. ROBERTS AND E. ALDOUS, *J. Bacteriol.*, 57 (1949) 363.
17 G. E. STAPLETON, A. J. SBARRA AND A. HOLLAENDER, *J. Bacteriol.*, 70 (1955) 7.
18 T. ALPER AND N. E. GILLIES, *J. Gen. Microbiol.*, 18 (1958) 461.
19 T. ALPER AND N. E. GILLIES, *J. Gen. Microbiol.*, 22 (1960) 113.
20 T. ALPER, *Int. J. Rad. Biol.*, in the press.
21 T. ALPER, N. E. GILLIES AND M. M. ELKIND, *Nature*, 186 (1960) 1062.
22 A. HOLLAENDER, G. E. STAPLETON AND F. L. MARTIN, *Nature*, 167 (1951) 103.