RADIATION RESISTANCE AND GENETIC SEGREGATION IN A LARGE CELL POSSIBLY POLYPLOID STRAIN OF *ESCHERICHIA COLI*[1]

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The concept that the killing of bacterial cells by radiation may involve nuclear damage seems first to have been formulated by Henri (1914) on the basis of her studies with different regions of the ultraviolet spectrum separated by filters. Reasoning that sublethal exposures to radiation might therefore produce heritable modifications, she successfully isolated both stable and unstable variants of *Bacillus anthracis* differing in pathogenicity and morphology from the survivors of ultraviolet radiation. Although Rahn (1929) first formulated the hypothesis that inactivation was due to the induction of lethal mutations, Lea (1947) was the most active proponent of this hypothesis. Lea interpreted the mass of data collected by himself and his associates on the inactivation of *Escherichia coli* by a variety of different radiations as being due to the induction of lethal mutations among some 250 genes each 12 mμ in diameter. Much evidence, briefly reviewed by Zelle (1955), has accumulated in recent years indicating that the inactivation of bacteria by radiation is not due to recessive lethal mutations and possibly is not due to nuclear damage at all. Perhaps most important are Witkin's (1951) observations of a correlation between the ratio of uninucleate and multinucleate cells and the ratio of intact to sectored lactose negative mutants in *E. coli* following ultraviolet radiation and a correspondence between the modal number of nuclei per cell and the modal size of mutant sector. No such relationships would be expected with survival ratios as low as 10^{-3}, as in her experiments, for the probability of surviving cells having more than one viable nucleus would be very low if inactivation were due to nuclear damage. In similar experiments, Newcombe (1953) observed sectored mutant colonies following γ-radiation and also failed to observe a decrease in the proportion of sectored mutant colonies with an increase in ultraviolet dose as would be expected on the nuclear damage hypothesis.

In contrast, much evidence has accumulated indicating that inactivation by both high energy and low energy radiations of Neurospora conidia results from nuclear inactivation (Atwood, 1950; Norman, 1954). Similarly, the sensitivity of yeasts to both high and low energy radiations has been shown to be a function of the degree of ploidy (Latarjet and Ephrussi, 1949; Tobias, 1950; Lucke and Sarachek, 1953; Uretz, 1955).

A stable large cell possibly polyploid strain of *E. coli* isolated following treatment with camphor vapors (Ogg and Zelle, 1957) has been found to be significantly more resistant to both high and low energy radiations and to display quite different inactivation kinetics than the strain from which it was derived. The present paper is concerned with this difference in radiation resistance and kinetics, with possible genetic inferences of these data, and presents evidence that segregation of cell size and radiation resistance occurs in prototroph progeny obtained by genetic recombination in crosses involving the stable large cell strain.

MATERIALS AND METHOD

The isolation of the large cell strain P6 from *E. coli* strain 82/r after treatment with camphor vapors has been described (Ogg and Zelle, 1957). *E. coli* strain IMN-64 (designated hereafter as 64), another auxotrophic mutant of E. coli strain B/r was obtained through the courtesy of Dr. P. D. Skaar and was utilized in the genetic recombination tests along with *E. coli* strains 58-161 and W1177, derivatives of *E. coli* strain K-12 obtained through the courtesy of Dr. J. Lederberg.

Unless otherwise indicated, cells for radiation were grown for 15 to 18 hr in aerated nutrient broth cultures at 37 C. The cell suspensions were

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washed twice and resuspended in M/15 phosphate buffer, pH 6.8. For X-ray and γ-ray experiments, the washed suspensions were saturated with oxygen by bubbling at ice bath temperature for 15 min. Survival assays were made on the surface of nutrient agar plates which were counted after 24 to 48 hr of incubation at 37 C.

The X-ray source was a General Electric Maxitron 250 unit operated at 250 kvp and 30 ma with 3 mm added aluminum filtration. X-ray dosage was determined with a 250 r thimble ionization chamber of a standardized Victoreen dosimeter. In the γ-ray experiments, the cell suspensions were irradiated by means of a 300-curie cylindrical cobalt-60 source delivering 1.57 kr per min. The irradiations were performed at the Biology Division of the Oak Ridge National Laboratory through the courtesy of Dr. Alexander Hollaender.

RESULTS

X-ray and γ-ray inactivation of strains 82/r and P6. Semilogarithmically plotted survival curves following X-ray and γ-ray irradiation of 82/r and its large cell derivative P6 are presented in figures 1 and 2 respectively. The curves are the average of six replicated experiments. The dotted lines are extrapolates to zero dose of the presumed linear portion of the survival curve which Atwood and Norman (1949) have shown to yield estimates of the average target number per cell in their multitarget interpretation of sigmoidal inactivation curves. With both X-rays and γ-rays, 82/r displays exponential survival whereas P6 is markedly more resistant and also has a sigmoidal inactivation curve. Since 82/r has essentially the same resistance to radiation as the radiation resistant *E. coli* strain B/r, P6 is an extremely radiation resistant strain.

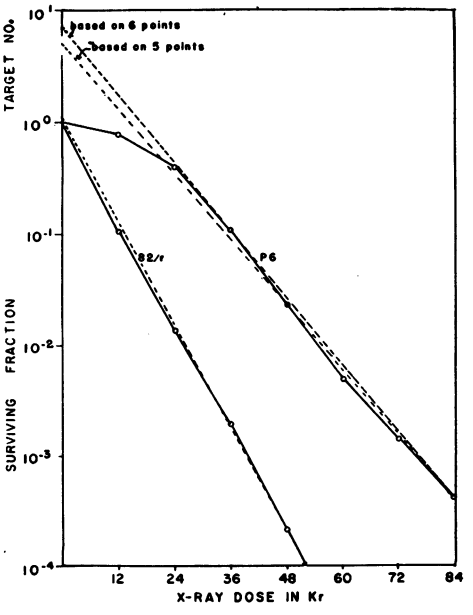

Figure 1. X-ray survival curves for *Escherichia coli* strains 82/r and P6.

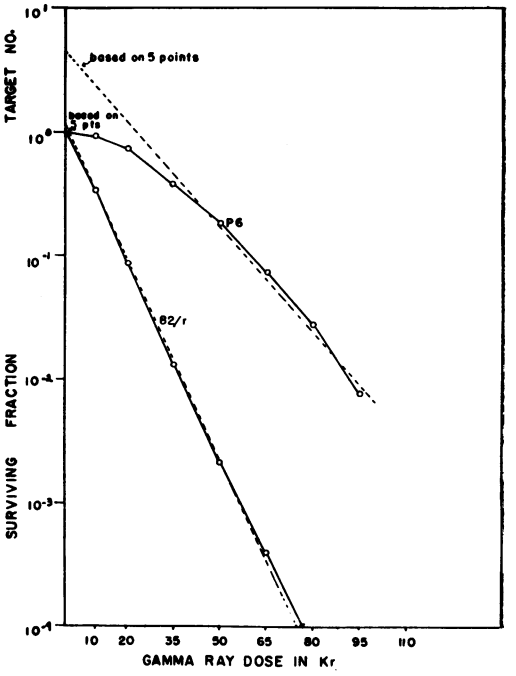

Figure 2. γ-ray survival curves for *Escherichia coli* strains 82/r and P6.

Lucke and Sarachek (1953) have shown that the inactivation curves of haploid, diploid, triploid, and tetraploid yeasts yield extrapolated estimates of the target number of 1, 2, 3, and 4 respectively. It was of interest, therefore, to determine the estimated target number on the basis of the multitarget hypothesis (Atwood and Norman, 1949). Their extrapolation procedure is statistically inefficient since it utilizes information obtained from only a relatively small portion of the entire radiated population. Furthermore, the points which may be considered to be linear cannot always be determined accurately and the inclusion of one or two additional points at the beginning of the linear portion of the curve can

sometimes greatly influence the value of the extrapolate obtained. To circumvent these difficulties, the analytical method developed by Kimball (1953) which utilizes all of the points of the survival curves and has the added advantage of being completely objective was employed. The target numbers of about 2.5 for X-rays and 2.7 for γ-rays for P6 would suggest either diploidy and triploidy if it is wished to make this kind of inference.

However, the haploid sets of chromosomes making up a diploid or polyploid nucleus do not retain their separate identity. Rather, all of the chromosomes intermingle in the nuclear sap and constitute a single nucleus. Hence, the correspondence between the target extrapolate and the degree of ploidy which has been observed in yeast may well be only coincidence and without real biological significance.

The model proposed by Tobias (1950) seems much more appropriate for interpretation of inactivation curves obtained from a polyploid series. In this model, a haploid may be inactivated as a result of inactivation of any one of a number of sites on the haploid chromosome complement whereas for diploids or higher polyploids to be inactivated, all of the homologues at some one site must be inactivated. The equations are:

$$N/No = e^{-naD} \qquad (1)$$

Haploid survival curve

$$N/No = [1 - (1 - e^{-aD})^m]^n \qquad (2)$$

Polyploid survival curve

where N/No = the survival ratio; n = the number of sites per chromosome set; m = the degree of ploidy; a = the average sensitivity per site; D = the dose. For simplicity, a is assumed to be the same for all sites and in both haploid and diploid or polyploid organisms. Where m, the degree of ploidy, is known, the haploid and polyploid survival curves yield estimates of n and a. In the case of the 82/r and P6 data, however, the values of m, n, or a are not known. However, reasonably accurate exponential inactivation curves for 82/r of the form $N/No = e^{-kD}$ were available where $k = na$ in equation (1). By assuming a reasonable value for n, the corresponding value of a $(=k/n)$ can be computed and these values of n and a can then be used to compute values of N/No for different values of

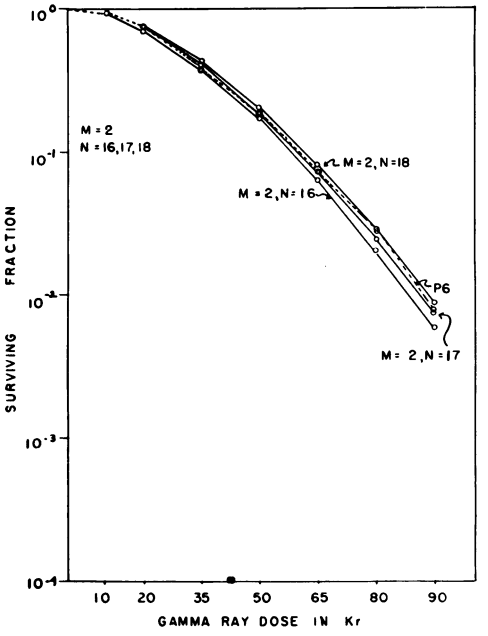

Figure 3. Comparison of actual and calculated γ-ray survival curves for P6 based on the Tobias model.

m from equation (2). Thus by using the parameters from the almost certainly haploid 82/r survival curve and substituting them into equation (2), a family of curves for different combinations of n and m were plotted and compared with the experimental survival curves of P6. Such curves were plotted for m = 2, 3, 4, and n = 4, 6, 8, 12, 16, 17, 18, 20, and 24. The calculated gamma ray survival curves for m = 2 (diploidy) and n = 16, 17, and 18 are shown in figure 3. The m = 2, n = 17 survival curve is an excellent fit to the experimentally observed P6 inactivation curve. None of the other calculated curves approach this close a fit. Calculated X-ray survival curves for m = 2, n = 12 and m = 3, n = 6 which most closely corresponded to the observed P6 survival curve are plotted in figure 4. Since the survival curve for P6 tails off slightly, the dotted extension line was drawn to help adjust the closeness of fit. It appears that the m = 2, n = 12 curve most closely approximates the observed P6 curve but obviously, no statistically significant choice can be made between them. It appears therefore that a hypothesis of diploidy with some 12 to 18 sites per haploid chromosome set would satisfactorily explain the

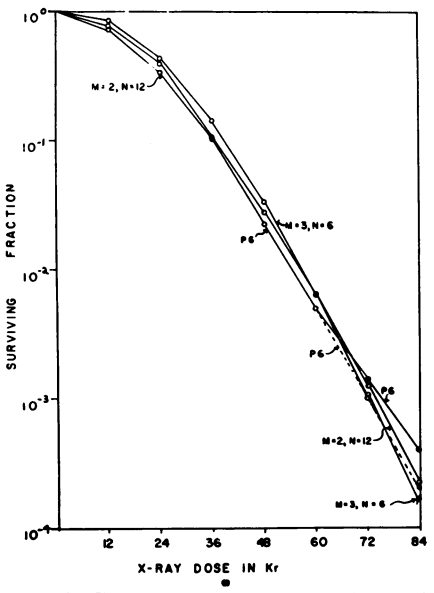

Figure 4. Comparison of actual and calculated X-ray survival curves for P6 based on the Tobias model.

observed inactivation curves on the Tobias hypothesis. Thus, the analysis of the survival curves obtained from P6 and 82/r with X-rays and γ-rays yield further evidence of an indirect nature that P6 could be a diploid derivative of 82/r. However, it must be emphasized that such analyses cannot of themselves supply critical proof of a diploid or polyploid genetic constitution.

Resistance to ultraviolet inactivation. Evidence concerning the comparative resistance of P6 and 82/r to ultraviolet irradiation is not as extensive as for the ionizing radiations. When P6 and 82/r are exposed in thin, nonabsorbing suspension to equal doses of incident energy from a 2537A germicidal lamp, the survival curves are almost coincident. Since, however, P6 has about 3 times greater DNA and RNA content and dry weight than 82/r (Ogg and Zelle 1957), it would be expected that the ultraviolet absorption coefficient per cell would be correspondingly greater for P6 than for 82/r. This has been found to be the case (Zelle and Alexander 1957, and *unpublished data*) with the absorption coefficient of P6 being from about 1.6 to nearly 6 times as great as that for 82/r at different stages of the growth cycle. For stationary phase cells, the ratio is about 2. Thus if one were to convert the survival curves obtained from exposure to equal amounts of incident energy to an absorbed energy basis by the use of such absorption coefficients, P6 would be about 2 times as resistant to ultraviolet. This has been confirmed in tests with monochromatic 2650A ultraviolet where the organisms were exposed in thick suspensions which absorbed all of the incident energy after the method of Hollaender and Claus (1936). The energy absorbed per cell is calculated from the total energy incident on the cell suspension in which the total cell content is known. Estimates of the 37 per cent survival doses indicate that P6 is about 2.5 times as resistant as 82/r, which is in reasonably good agreement with the estimate of 2.0 made above.

Segregation of colony morphology, cell size, and γ-ray resistance in genetic recombination tests. Calef (1954) discovered that strains of *E. coli* B and B/r could be crossed with strains of *E. coli* K-12 if the latter carried the F^+ compatibility factor (Cavalli *et al.* 1953), the *E. coli* B and B/r strains acting as F^-. Conversion of *E. coli* B and B/r from F^- to F^+ was accomplished by DeHaan (1954) by growing in mixed culture with F^+ strains. Since 82/r and P6 carry a purineless mutation, the prototroph selection technique (Lederberg 1947) could be utilized in recombination tests. The comparative recombinational analysis of 82/r and P6 has been hampered by the lack of suitably marked strains. Additional markers are being added to substrains so that more detailed genetic analysis will soon be possible. The present paper therefore will be concerned only with preliminary data on the frequency of prototroph formation and the segregation of colony morphology, cell size, and γ-ray resistance in prototrophs formed from crosses involving 82/r and P6.

Strain 64, also a derivative of *E. coli* strain B/r, was chosen as the common parent to which 82/r and P6 were crossed for this study. Strain 64 carries a mutation causing a multiple requirement of tryptophan, tyrosine, phenylalanine, and *p*-aminobenzoic acid, is T1 phage resistant (V_1^r), and is characterized by cell size, colony morphology, and γ-ray resistance almost identical to that of 82/r. All three strains, 82/r, P6, and 64, were converted to F^+ by growing in mixed culture with a culture of W1177F^+ which had previously been made F^+ by growing in mixed culture with 58-161F^+. The crosses were made on M9 medium (Anderson, 1946) containing, in addition to inorganic salts, NH_4Cl and

TABLE 1
Frequency of prototroph formation in reversed crosses of strains P6 and 82/r with 64

Cross	Back-Mutants* per Cells	Prototrophs per 10^8 P6 or 82/r Cell
82/rF$^+$ x 64F$^-$	10	60
64F$^+$ x 82/rF$^-$	4	65
P6F$^+$ x 64F$^-$	3	784
64F$^+$ x P6F$^-$	6	263

* Back-mutants are the sum for both parental suspensions.

glucose as nitrogen and carbon sources. The technique utilized in the crosses was essentially that described by Lederberg (1947).

The frequency of prototroph formation in reverse crosses of P6 and 82/r with strain 64 are presented in table 1. It is readily apparent that the frequency in crosses involving P6 as either the F$^+$ or F$^-$ parent is significantly higher than in comparable crosses with 82/r. It will also be noted that control platings exhibit back-mutant colonies which might be isolated as prototroph recombinants on the cross plates. With the higher fertility exhibited by P6, this possibility is not serious. However, the proportion of presumed prototroph segregants in crosses involving 82/r which could be back mutations of either 82/r or 64 is higher than desirable and some caution must be exercised in the interpretation of such data. In the present case, since both 82/r and 64 exhibit similar colony morphology, cell size and γ-ray resistance, no segregation of these characteristics was observed so that back mutants are unimportant.

The significantly higher frequency of prototroph formation in crosses involving P6 has been confirmed in crosses of 82/r and P6 with 58-161, W1177F$^+$ and in crosses between derivatives of 82/r and P6 with derivatives of strain 64. If in the process of genetic recombination in *E. coli*, a complete zygote containing both parental genomes is formed (Nelson and Lederberg, 1954), this increased frequency is compatible with the hypothesis that P6 may be a diploid or polyploid derivative of 82/r since in this case, a polyploid zygote would be formed and prototrophs could originate by whole chromosome segregation as well as by gene recombination by crossing over, the only mechanism whereby prototrophs could be formed in crosses between haploid strains 82/r and 64.

If this were actually the case, one would expect to find only haploid prototrophs in crosses between 82/r and 64 whereas both haploid and diploid or polyploid prototrophs might be expected in crosses between P6 and 64. Further, if the premise that cell size and radiation resistance are functions of the degree of ploidy were also correct, only small cell, radiation sensitive prototrophs should be obtained from crosses of 82/r with 64 whereas some large celled, radiation resistant prototrophs should be found in the progeny of crosses between P6 and 64. Accordingly, a number of prototrophs derived from crosses of P6 and 82/r previously classified for $V_1{}^r$ were further classified for cell size by microscopic examination. The results (table 2) show that, as expected, all of the prototrophs derived from crosses of 82/r with 64 are characterized by small cells similar to 82/r whereas prototrophs derived from crosses of P6 and 64 are of two types having either large cells like P6 or small cells like 82/r. The results also show that the F polarity which influences segregation of non-selected markers (Hayes, 1953; Cavalli *et al.*, 1953) affects the segregation of $V_1{}^r$ and cell size with the majority of prototrophs resembling the F$^-$ parent.

Since it was found that cell size segregated in genetic crosses, it seemed likely that the high resistance to radiations characteristic of the large cell P6 strain would display similar segregation. Hence, to determine if colony morphology, cell size and radiation resistance segregated together in the prototrophs, a number of prototrophs were isolated from the various crosses and classified with respect to these three characteristics. In determining the gamma ray resistance, prototrophs which had been freed of parental contamination by streaking on minimal agar

TABLE 2
Cell size of prototrophs

Cross*	P6 Type			82/r Type		
	$V_1{}^s$	$V_1{}^r$	Total	$V_1{}^s$	$V_1{}^r$	Total
64F$^+$ x P6F$^-$	85	5	90	7	3	10
64F$^-$ x P6F$^+$	13	26	39	8	53	61
64F$^+$ x 82/rF$^-$	0	0	0	57	22	79
64F$^-$ x 82/rF$^+$	0	0	0	12	68	80

* In all crosses, $V_1{}^r$ was carried by 64F$^-$ or 64F$^+$.

were inoculated into liquid M9 medium and incubated 15 to 18 hr at 37 C under aeration. The cell suspensions for irradiation were prepared by diluting the cultures 1 to 100 in M/15 phosphate buffer, pH 6.8, and saturating with oxygen by bubbling 15 to 20 min at ice bath temperature. Two ml aliquots were placed in glass stoppered volumetric pyrex tubes and exposed to γ-rays in the cobalt-60 source described earlier. Seven cultures could be irradiated simultaneously. Since figure 2 indicates a pronounced difference between P6 and 82/r (and/or 64) in γ-ray resistance, only three doses were given, 20, 40 and 60 kr.

Although a relatively limited number of independently isolated prototrophs were irradiated in this manner, the results obtained (table 3) were sufficient to establish a complete correlation between colony morphology as assessed by internal granularity, cell size, and γ-ray resistance. Thus with 2 exceptions, all prototrophs irradiated could be classified as coarsely granular, large cell, radiation resistant like P6 or finely granular, small cell, radiation sensitive like 82/r or 64. The evidence obtained from stained smears and examination of colonies of the survivors to radiation indicated that the two mixed intermediate prototrophs were either mixed cultures derived from two prototrophs of each of the above types or else that the originally occurring prototrophs had segregated into stable subcultures of these two types since the culture was obviously mixed and since the proportion of coarsely granular colonies increased on the plates from larger gamma ray doses.

The occurrence of the two mixed intermediate cultures does not obscure the primary conclusion that prototrophs from crosses of 82/r with 64 are all small cell and radiation sensitive types whereas prototrophs from crosses of P6 with 64 are of two types resembling the two parents with respect to cell size and γ-ray resistance. As indicated in table 1, a small proportion of the prototrophs derived from crosses involving P6 could actually be back-mutants of P6 although the frequency of such back-mutants in relation to the frequency of prototroph formation is so small as to make the possibility that the 25 large cell prototrophs selected for γ-ray tests were all back-mutants of P6 highly improbable to say the least. Even so, the 15 V_1^r radiation resistant prototrophs provide further evidence that this could not be the case since coincident mutations to purine-independence and T1 phage resistance would be necessary for such types to be derived from P6 directly. Similarly, the evidence is strong that the occurrence of small cell, radiation sensitive prototrophs is bonafide since the occurrence of small cell V_1^r, radiation sensitive prototrophs from P6 would require loss of the large cell characteristic coincidental with mutation to V_1^r. As reported earlier (Ogg and Zelle, 1957) P6 has remained remarkably stable through many generations of continuous growth and through repeated transfers on stock culture agar. The occurrence of radiation sensitive prototrophs in the P6 x 64 crosses which are V_1^s would require mutation of the multiple nutritional factor in strain 64 along with mutation from V_1^r to V_1^s. Thus despite the handicap of prototroph selection based on only single factor nutritional requirements, the evidence seems convincing that segregation of cell size and γ-ray resistance does occur in prototrophs derived from P6 crossed with 64.

TABLE 3

Classification of selected prototrophs as to cell size, T1 phage resistance and γ-ray resistance

Cross	Prototroph Type				
	Large cell, radiation resistant		Small cell, radiation sensitive		Mixed, intermediate
	V_1^r	V_1^s	V_1^r	V_1^s	V_1^r
82/rF$^+$ x 64F$^-$	0	0	9	4	0
64F$^-$ x 82/rF$^+$	0	0	6	2	0
P6F$^+$ x 64F$^-$	6	4	2	6	0
64F$^-$ x P6F$^+$	9	6	10	5	2

* In all crosses, V_1^r was carried by 64F$^-$ or 64F$^+$.

DISCUSSION

The evidence derived from the comparative radiation sensitivity of 82/r and P6 along with that supplied by the genetic recombination tests fortify the evidence presented by Ogg and Zelle (1957) which is in large part compatible with the hypothesis that P6 may be a diploid or polyploid derivative of 82/r. Evidence supporting this hypothesis includes comparative DNA and RNA content, dry wt, microscopical counts of relative number of nuclei per cell, and in the present paper, the increased radiation resistance and

form of the survival curve following X- and γ-rays. The significantly greater frequency of prototroph formation and the segregation of cell size and γ-ray resistance amongst prototrophs of crosses involving P6 can also be added. Opposing this array of evidence are the results of studies of induced mutations to T1 phage resistance and streptomycin resistance which indicate that, unless a high rate of mitotic segregation either by crossing over or a disjunctional division of chromosomes occurs, P6 could not be considered a diploid or polyploid. The possibility that irradiation induces a high rate of mitotic segregation in P6 has not yet been adequately tested although what evidence has been obtained thus far suggests that it does not. Obviously, the question as to the true genetic nature of the large cell P6 strain can only be resolved from detailed genetic analysis of prototrophs derived from crosses involving P6 and 82/r.

The possibility that the large cell size and radiation resistance of P6 is due to a point mutation induced by treatment with camphor vapors has not yet been considered. Lieb *et al.* (1955) have observed apparently independent segregation of cell shape and nuclear morphology in recombination studies of *E. coli* strain K-12S (sensitive to phage λ) and *E. coli* strain C. The evidence derived from segregation of cell size and γ-ray resistance in recombination experiments is compatible with the mutation hypothesis since the prototrophs segregate into two distinct categories resembling the parental types with no intermediate types. The ratio of prototrophs of the two types is influenced by the F polarity just as is segregation of any nonselected marker. If true, this would be an interesting mutation affecting the mechanisms regulating cell size and nuclear number in *E. coli*. Furthermore, since other stable large cell strains have been isolated following camphor vapor treatment, it would appear that this particular mutation would be rather specifically induced by camphor vapor treatment, a situation without parallel so far as the authors are aware.

The segregation of radiation resistance and cell size in prototrophs has some interesting interrelationships with the question of the nuclear or non-nuclear nature of lethal radiation effects and with the question of complete or partial zygote formation in genetic crosses. Whereas Nelson and Lederberg (1954) have evidence indicating transfer of a complete genome from the F^+ parent, Wollman and Jacob (1955) found that in crosses of F^- with Hfr, the contribution of the Hfr parent is a variable length chromosome segment in which the genes are transferred sequentially. The sequential transfer of a chromosome segment has been confirmed by Fuerst *et al.* (1956) in studies of the loss of ability to transfer genes due to P^{32} decay, and by Skaar and Garen (1956) who in addition have shown that the order of gene transfer is dependent on the particular Hfr strain employed and that sequential transfer occurs in spontaneous exconjugants supporting the view that normally, incomplete genomes are transferred. Lederberg (1956) has shown that recombination involves conjugal pairing, that both Hfr and F^- exconjugants usually survive, and that recombinants segregate only from the F^- exconjugant. Photographs of presumably conjugating pairs suggest that cytoplasmic as well as gametic transfers could occur but nothing is known of the extent of cytoplasmic interchange if any during conjugation. In any case, it seems difficult to interpret the segregation of radiation resistance in prototrophs from reversed crosses with respect to F polarity into two classes resembling the parents with no intermediates as non-nuclear. It does not necessarily follow that the lethal radiation damage is also nuclear but the postulated cytoplasmic differences presumed to be responsible for differences in resistance to radiation would clearly be under nuclear control.

Assuming for the moment that P6 is polyploid and that lethal radiation damage involves inactivation of homologous chromosomal sites as postulated by Tobias (1950) for yeast, the segregation of cell size and radiation resistance would be most easily understood on the basis of complete genome transfer. If incomplete genomes are transferred, the simplest interpretation would appear to be that the preferentially transferred chromosomal segment carries a camphor-induced mutant gene which causes the increased cell size, DNA content and radiation resistance characteristic of P6. In this case, the site of lethal radiation damage would appear to be cytoplasmic since the radiation resistant prototroph would be haploid and differ from the radiation sensitive prototrophs from the same cross only by the substitution of this single gene. These implications add interest and importance to the resolution of the question of the genetic nature of P6.

SUMMARY

Comparative tests were made of the resistance to radiation of *Escherichia coli* strain 82/r and a stable, large cell, possibly polyploid derivative, P6, produced by exposure of 82/r to camphor vapors. P6 was markedly more resistant to X-rays, γ-rays and ultraviolet.

Whereas 82/r exhibits exponential survival curves following X-rays and γ-rays, P6 is characterized by sigmoidal or multihit survival curves. Analyses of the multihit curves yield estimates of the target number corresponding to those observed in polyploid series of yeasts. Interpretations in accordance with the hypothesis of Tobias (1950) are compatible with the hypothesis that P6 may be a diploid derivative of 82/r.

Strains 82/r, P6 and 64, all derived from *E. coli* strain B/r may be converted to F^+ and utilized in genetic crosses. P6 displays a significantly higher frequency of prototroph formation in crosses with 64 than does strain 82/r, a result compatible with the hypothesis that P6 may be diploid or polyploid since in that event prototrophs could be formed by whole chromosome segregation in addition to formation by crossing over between auxotrophic mutations.

Prototrophs derived from crosses of 82/r with 64 made reciprocally with respect to the F factors are all characterized by finely granular colony morphology, small cell size similar to that of both parents, and γ-ray sensitivity like that of 82/r and 64. By contrast, prototrophs derived from reversed crosses of P6 with 64 are almost entirely of two types: small cell, radiation sensitive type like 82/r and 64, and large cell, γ-ray resistant type similar to P6.

Segregation of radiation resistance and cell size in reversed crosses is influenced by F polarity in a manner similar to unselected markers in general.

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