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ANALYSIS OF A GENE CONTROLLING CELL DIVISION AND SENSITIVITY TO RADIATION IN *ESCHERICHIA COLI*

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ABSTRACT

ADLER, HOWARD I. (Oak Ridge National Laboratory, Oak Ridge, Tenn.), AND ALICE A. HARDIGREE. Analysis of a genetic locus controlling cell division and sensitivity to radiation in *Escherichia coli*. J. Bacteriol. **87**:720–726. 1964.—Radiation sensitivity in *Escherichia coli* is under control of genes that are transferred during conjugation between donor and recipient strains. Conjugation experiments establish that one of these genes occupies a locus on the *E. coli* K-12 linkage map between the genes controlling ability to utilize lactose and galactose. It affects sensitivity to both ionizing and ultraviolet (2,537 A) radiation. A strain possessing a mutation at this locus fails to show increases in resistance to ionizing radiation during late lag and early log phases, and increases in resistance when grown to stationary phase in a glucose-containing complete medium. The primary effect of the mutation at this locus may be an interference with the mechanism by which cells form cross plates. Cells of the mutant form long, nonseptate filaments when grown after exposure to ionizing radiation. The filaments do not give rise to macrocolonies. Pantoyl lactone, an agent that initiates cross plate formation, allows the filaments to divide normally and produce macrocolonies. When plated after irradiation on complete medium containing pantoyl lactone, the survival of the mutant is greatly increased.

The sensitivity of *Escherichia coli* K-12 to ionizing and ultraviolet radiation is influenced by several genetic loci that are transferred as chromosomal elements during conjugation (Adler and Copeland, 1962). The conjugation system has been used to study individual loci affecting the radiation sensitivity of *E. coli* in detail (Howard-Flanders et al., 1962; Howard-Flanders, Simson, and Theriot, Genetics *in press;* Rörsch, Edelman and Cohen, 1963; van de Putte, Westenbroek, and Rörsch, *in preparation*). These reports have been primarily concerned with sensitivity to ultraviolet radiation. In this paper, we present genetic and physiological data describing a locus in *E. coli* K-12 that influences sensitivity to ionizing radiation. The effect of this locus on ultraviolet-radiation sensitivity has been discussed (Howard-Flanders et al., *in press*). The data suggest that the locus is involved in the formation of cross plates—an important step in the division cycle of bacterial cells.

[1] Operated by Union Carbide Corp. for the U.S. Atomic Energy Commission.

MATERIALS AND METHODS

Three strains of *Escherichia coli* K-12 were used in these experiments. The HfrH strain, a high-frequency donor of genetic material, was obtained from Roy Curtiss of this Laboratory. AB1157 and AB1899 are F^- (recipient) strains obtained from Paul Howard-Flanders. AB1899 is an X-ray- and ultraviolet-sensitive mutant obtained by P. Howard-Flanders after exposure of AB1157 to ultraviolet light. Characteristics of these strains are presented in Table 1. The markers are arranged in the order in which they are transferred by HfrH.

Complete and synthetic media, techniques for mating experiments, purification of recombinants, X-ray survival curves, and X-ray assay points were described previously (Adler and Copeland, 1962). Since early log-phase cells were found to be sensitive to ice-bath temperatures, irradiations were performed at 16 C (Sherman and Albus, 1923). Glucose, when used, was added to the complete liquid medium to a final concentration of 0.5 or 1%. Pantoyl lactone (DL-pantoyl lactone, Nutritional Biochemicals Corp., Cleveland, Ohio), when used, was added to complete agar medium to a final concentration of 0.08 M.

Heat-inactivation experiments were performed by diluting small quantities of cell suspensions into a 0.067 M phosphate buffer (pH 6.8) pretempered at 54 C. Samples were removed at intervals and diluted quickly in buffer at 25 C before plating.

Ribonucleic acid (RNA) determinations were performed on trichloroacetic acid extracts accord-

TABLE 1. *Characteristics of Escherichia coli K-12 strains*

Strain	Characteristic*					
	TL	*Pro*	*Lac*	T6	*Gal*	*Str*
HfrH.....	+	+	+	S	+	S
AB1157.....	−	−	−	R	−	R
AB1899.....	−	−	−	R	−	R

* Abbreviations: threonine-leucine, *TL;* proline, *Pro;* lactose, *Lac;* bacteriophage T6, T6; galactose, *Gal;* streptomycin, *Str.* A plus sign indicates ability to synthesize for the amino acid characteristics and ability to utilize as sole carbon source in the case of the sugars. S denotes sensitivity; R denotes resistance.

ing to the orcinol colorimetric method proposed by Volkin and Cohn (1954).

Microscopic observation of cell division and colony formation was made by spreading appropriate dilutions of cells onto a thin layer of complete agar medium on a microscope slide maintained at 37 C. Agar blocks were placed on the ends of the slide to retard drying, and a cover slip was placed over the center of the slide to allow observation of the dividing cells with an oil immersion lens. Photographs were taken on Kodak Panatomic-X 35-mm film, making use of the 97× Bright M phase objective of an AO Phasestar microscope. Köhler illumination and a Wratten B58 filter were used.

All figures and tables represent results averaged from three or more experiments unless otherwise noted.

RESULTS

Many strains of *E. coli* become resistant to ionizing radiation if grown to stationary phase in a glucose-containing complete medium (Stapleton and Engel, 1960). The mutant AB1899 failed to show this effect. The parental culture, AB1157, showed a typical response (Fig. 1). The failure of AB1899 to show this effect is not due to an inability to utilize the sugar, because this organism produces as much acid from glucose as does the parental culture. To analyze the nature of the genetic alteration that led to the failure of ability to show this "glucose effect," *E. coli* AB1899 was used as the recipient strain in a cross with the high-frequency donor *E. coli* K-12 HfrH. This latter strain did show an increase in resistance if grown to stationary phase in a glucose-containing medium before irradiation. Survival curves for glucose-grown cultures of *E. coli* AB1899 and *E. coli* HfrH are shown in Fig. 2. Recombinants from the mating experiment were purified, grown in glucose-containing complete medium, and subjected to the X-ray assay technique. The recombinants either retained the "sensitivity" of AB1899 or acquired the ability to show the "glucose effect" characteristic of the HfrH donor (Fig. 3). No intermediate types were observed. By selecting recombinants known to have received certain biochemical traits from the HfrH parent and by scoring the frequency of unselected markers transferred to these recombinants, it was possible to construct a genetic linkage map including the region responsible for the ability to show the "glucose effect" (Table 2). It will be referred to as the "*lon*" locus as suggested by Howard-Flanders et al. (*in press*). In the experiment presented in Table 2, it was noticed that all recombinants that retained the sensitivity of AB1899 also retained the property of forming mucoid colonies on glucose-synthetic medium, characteristic of this organism. Therefore, an additional 100 $T^+L^+Str^R$ recombinants were

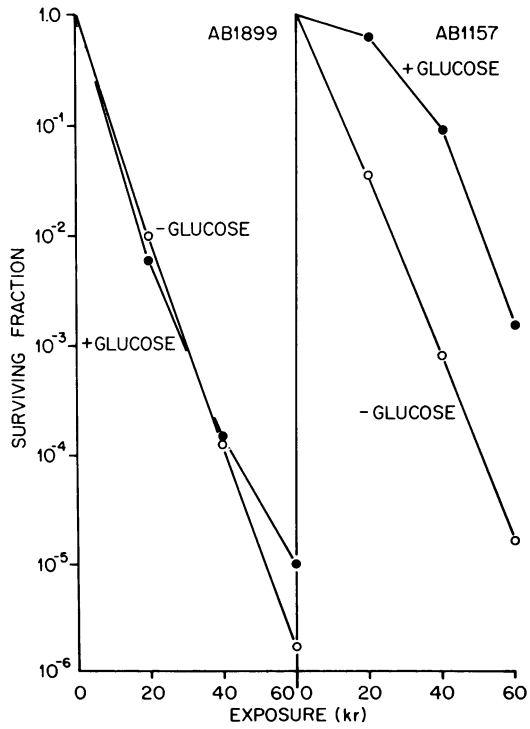

FIG. 1. *Survival curves for Escherichia coli K-12 AB1899 and AB1157 grown to stationary phase in complete medium with and without glucose.*

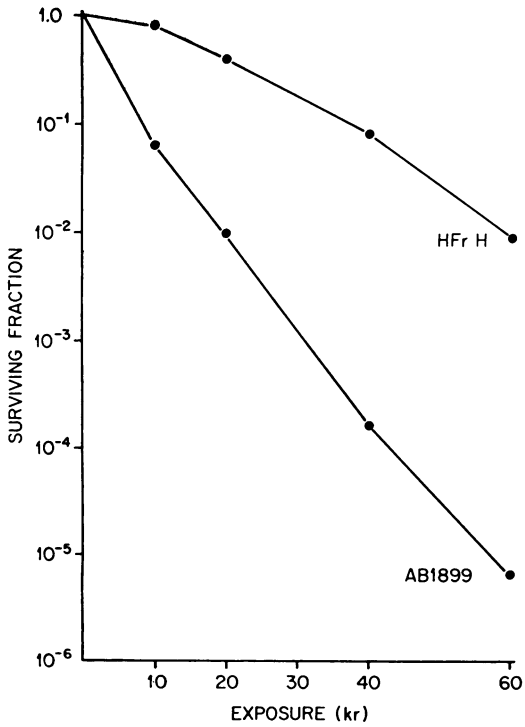

FIG. 2. *Survival curves for Escherichia coli K-12 HfrH and AB1899 grown to stationary phase in complete medium containing glucose.*

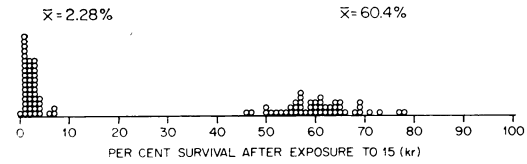

FIG. 3. *X-ray sensitivity of $T^+L^+Str^R$ recombinants grown in glucose-complete medium. Each circle represents a recombinant culture. Per cent survival was determined by plating on complete medium.*

scored for their ability to show the glucose effect and produce mucoid colonies. For all isolates, inability to show the glucose effect is correlated with the formation of mucoid colonies on glucose-containing synthetic media.

Since the mutant allele of *lon* prevents the development of resistance that occurs in many *E. coli* strains as they grow into stationary phase in glucose-complete medium, we were interested in determining whether it also affected the changes in X-ray sensitivity previously reported for lag- and log-phase cultures of *E. coli* (Stapleton, 1955). Each experimental point in Fig. 4 was obtained from averages of two survival curves. The changes in survival as a function of medium and cultural age at 20 kr reflect primarily changes in the shapes of survival curves. It can be seen that the mutant allele prevents the increase in resistance usually observed in the late lag-early log phase. In fact, there is a decrease in resistance in AB1899 during this period.

In addition to its effect on radiation behavior, the mutation at the *lon* locus resulted in the alteration of other properties. One of the most striking is the tendency of AB1899 to form nonseptate filaments. Microscopic observation of cell growth and division at 37 C allows us to make the following qualitative observations. (i) Unirradiated stationary-phase cells of the parent culture AB1157 grow and divide very regularly when transferred to an agar medium at 37 C. The microcolonies observable after 4 to 5 hr consist entirely of short cells (Fig. 5a). (ii) Unirradiated stationary-phase cells of the mutant AB1899 tend to form filaments which, after attaining several cell lengths, produce cells of

TABLE 2. *Genetic constitution of recombinants from the cross HfrH × AB1899**

Selected markers	Frequency (per cent) of unselected markers from HfrH					
	TL	*Pro*	*Lac*	*Lon*	T6	*Gal*
$T+L+Str^R$...	—	60	53	42	36	13
$Pro+Str^R$....	63	—	86	69	68	26

* Mating interrupted by vigorous agitation after 120 min.

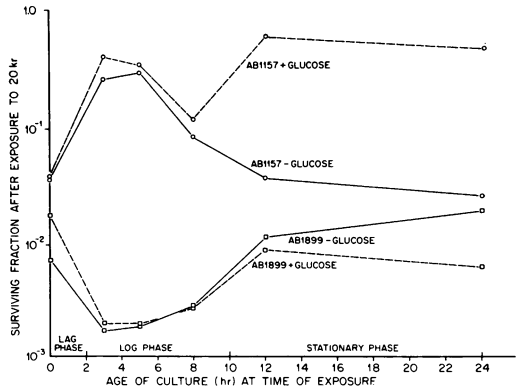

FIG. 4. *Changes in X-ray sensitivity of AB1899 and AB1157 grown at 30 C in complete medium with and without glucose.*

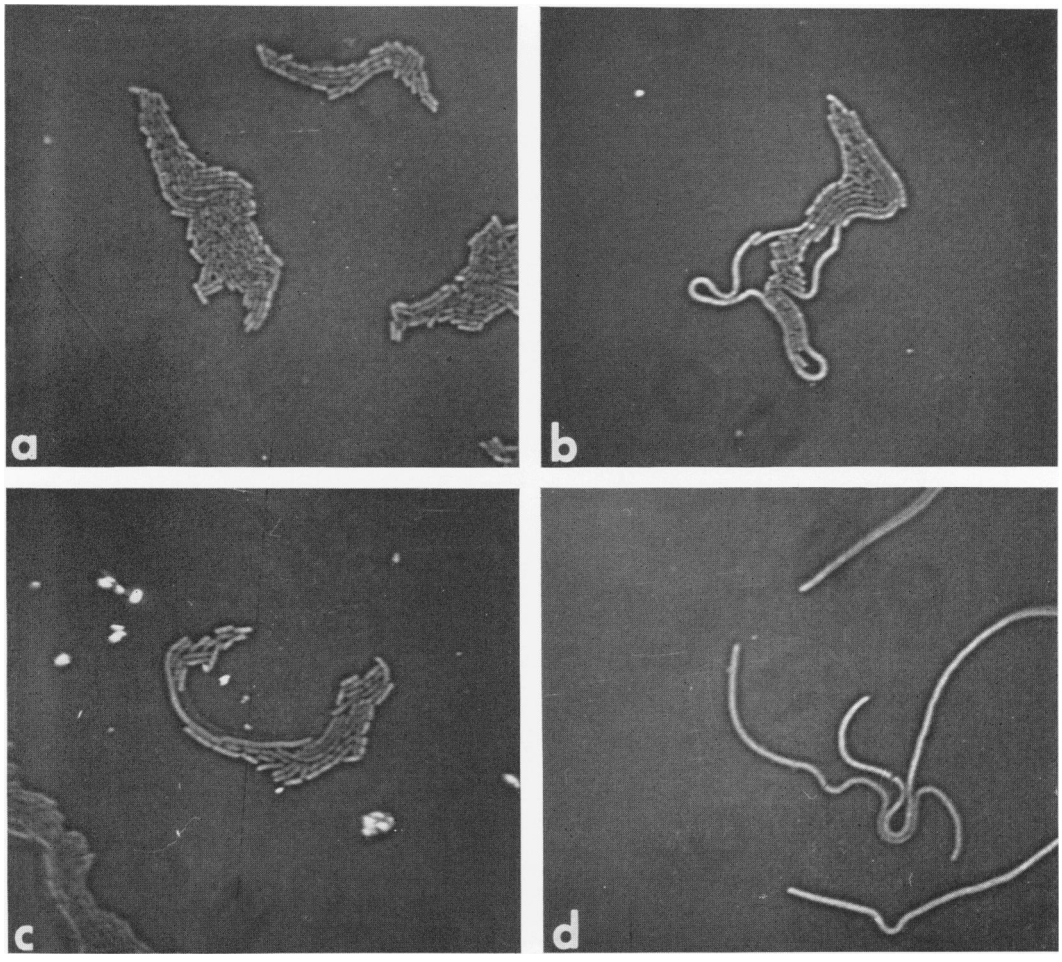

FIG. 5. *Microcolonies (4 to 5 hr at 37 C) grown from individual cells (×830). (a) Unirradiated AB1157. (b) Unirradiated AB1899. (c) Irradiated (10 kr) AB1157. (d) Irradiated (10 kr) AB1899.*

normal length (Fig. 5b). The microcolonies observable after 4 to 5 hr usually consist of a mixture of short, normal-appearing cells and nonseptate filaments. (iii) After exposure to 10 kr, most cells of the parent culture AB1157 begin to grow into filaments but many of these filaments produce cells of normal length, some of which then continue to divide normally and give rise to microcolonies (Fig. 5c). (iv) After exposure to 10 kr, most cells of the mutant AB1899 grow at a normal rate and form long, nonseptate filaments that fail to produce normal-length cells (Fig. 5d).

To establish whether the qualitative observations of the previous section are related to the difference in radiation sensitivity of AB1157 and AB1899 initially observed by plating methods, dose-response curves were obtained in which survival was estimated by counting macrocolonies after 24 hr and by scoring microscope slides for the frequency of filaments that had failed to give rise to normal cells after 4 to 5 hr of incubation at 37 C. The slides were coded so that the observer did not know what dose had been received by the cells being scored. Experiments of this type have been performed on stationary-phase cultures grown in glucose-containing complete medium and on early log-phase cultures grown in the absence of glucose. There was always good agreement between the survival

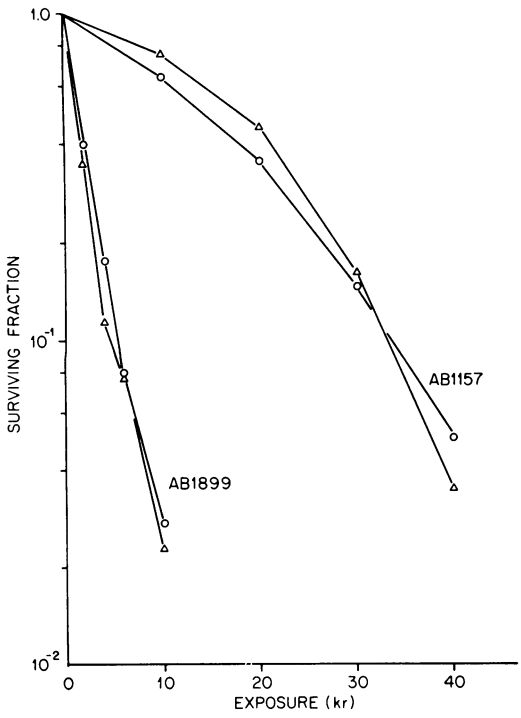

FIG. 6. *Survival curves for early log-phase culture of AB1899 and AB1157 estimated by macrocolony count after 24 hr (○) and by frequency of nonseptate filaments observed microscopically after 4 to 5 hr of growth at 37 C (△).*

estimated by the two methods. Figure 6 presents data averaged from two experiments on early log-phase AB1899 and AB1157 cultures.

Recent reports (Grula and Grula, 1962; van de Putte et al., *in preparation*) suggested that filament formation, induced by ultraviolet radiation, can be prevented or reversed by post-treatment with several agents. One of the most effective of these is pantoyl lactone. Figure 7 demonstrates the effect of pantoyl lactone in the postirradiation plating medium on the survival of AB1899 after exposure to X rays. A much smaller effect is observed for AB1157.

DISCUSSION

It has previously been observed that many strains of *E. coli*, when grown to stationary phase in a glucose-containing complete medium, become more resistant to ionizing radiation, ultraviolet radiation, heat, and hydrogen peroxide (Stapleton and Engel, 1960; Adler and Engel, 1961; Engel and Adler, 1961). Our attention was first drawn to the mutant culture AB1899 because of its failure to show this effect for ionizing radiation. It can be demonstrated, however, that glucose-grown AB1899 cells do show an enhanced resistance to heat inactivation (Fig. 8). Therefore, we suggest that glucose-induced resistance phenomena involve at least partially independent pathways and that the mutation present in AB1899 is relatively specific for radiation injury. The results of conjugation experiments suggest that the *lon* gene is between the loci controlling lactose and galactose utilization. This result agrees with that obtained by Howard-Flanders et al. (*in press*), who used different genetic donors and scored for sensitivity to ultraviolet radiation. *Lon* is closely linked to the T6 locus, but the available data do not allow us to distinguish between the order *Lac* . . . T6*lon* . . . *Gal* and *Lac* . . . *lon*T6 . . . *Gal*. The *lon* gene is within the large region known to contain several loci capable of influencing radiation sensitivity (Adler and Copeland, 1962).

The data from conjugation experiments also indicate that the failure to show the glucose

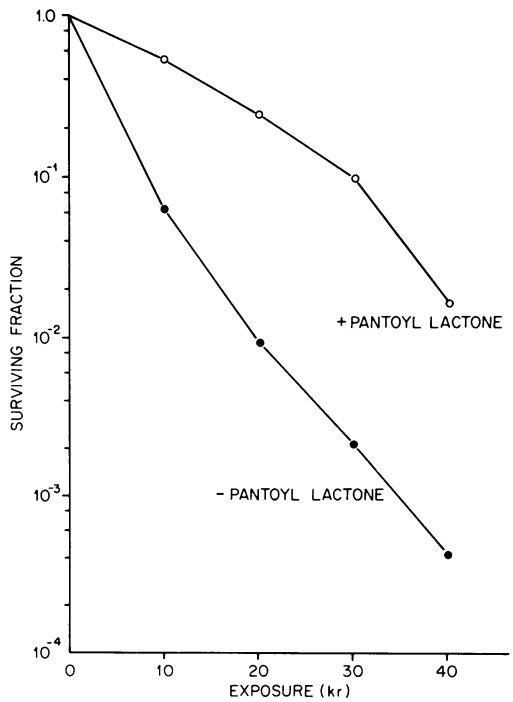

FIG. 7. *Effect of pantoyl lactone in the postirradiation plating medium on the survival of glucose-grown stationary phase AB1899.*

effect and the formation of mucoid colonies on synthetic media are reflections of one gene or of closely linked genes.

From the experiments described (Fig. 4), it can be seen that the *lon* gene has a more general effect than the determination of an organism's ability to show the glucose effect. It also has an effect on the changes in radiation sensitivity observed for *E. coli* cultures as they grow from lag phase into log phase. It has been suggested that this increase in resistance during the early phases of growth may be due to a multinuclear condition of cells at this time (Stapleton, 1955). Phase microscopic observation of AB1157 during late lag and early log phase does establish that it consists of long cells with several "nuclei," but the same observation is made for the radiation-sensitive cells of the mutant AB1899 at this stage of growth. Therefore, it seems unlikely that multinuclearity is responsible for the increase in resistance observed.

Large increases in RNA per cell have been reported to occur in *E. coli* during this same part of the growth cycle (Morse and Carter, 1949). We considered that this phenomenon might be related to the change in radiation resistance observed. In preliminary experiments, we observed approximately a fourfold increase in RNA per cell, but it occurs in both the radiation-resistant parent and the radiation-sensitive mutant strain and therefore cannot account for the increase in radiation resistance observed in AB1157 and other *E. coli* strains at this stage of growth.

In searching for the basic action of this gene that may account for its effect on ultraviolet- and ionizing-radiation sensitivity, our attention was drawn to the tendency of AB1899 to form long, nonseptate filaments under normal growth conditions. These may occasionally be observed in stationary-phase broth cultures. Their formation is greatly accentuated by radiation. Cell division cannot be considered complete unless a cross plate is formed.

This same phenomenon seems to be less radiation-sensitive in the parental culture AB1157. There are, however, in both cultures, changes in sensitivity of this mechanism during the cultural cycle. It is, however, clear from Fig. 6 that irradiated cells which fail to produce cross plates after 4 to 5 hr of incubation are finally inactivated and do not form macrocolonies. Howard-Flanders suggested that the unusual ultraviolet radiation sensitivity of AB1899 is due to an abnormality in the synthesis of cell-wall precursor or in the control of cell volume. We emphasize that it is particularly cross plate formation that is affected by ionizing radiation. The radiation damage may be at the genic level or at some later stage of cross plate synthesis and deposition.

Those cells which, as a result of irradiation, have lost the ability to form cross plates continue to elongate and synthesize cytoplasm and "nuclei" for several hours. This suggestion is made on the basis of phase-microscopic observation and is supported by quantitative data of Deering (1958). Apparently, many functions of the cell have not been damaged and are proceeding normally. Pantoyl lactone, an agent that stimulates cross plate formation, increases the probability that an X-rayed AB1899 cell will divide normally and give rise to a macrocolony (Fig. 7). van de Putte et al. (*in preparation*) recently made a comparable observation for ultraviolet-irradiated *E. coli* B. They attributed filament formation in this organism to a gene located between the loci controlling galactose fermentation and tryptophan synthesis.

The concentration of pantoyl lactone required for maximal effect is high (0.08 M) and does not seem to reflect a biochemical requirement. Its

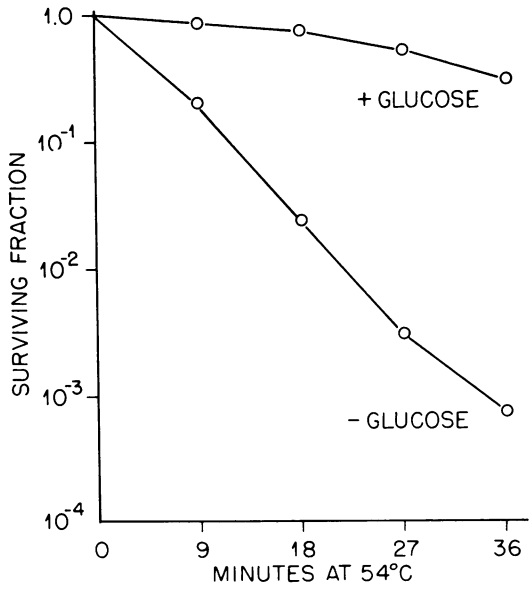

FIG. 8. *Heat sensitivity of AB1899 grown in complete medium with and without glucose.*

effect may be due to a contaminating material present in trace amounts or to some physical property of the compound. In any case, its presence in the postirradiation plating medium overcomes a large fraction of the radiation sensitivity introduced by mutation at the *lon* locus.

The *lon* gene functions in cross plate formation and cell division. Its effects on radiation behavior of cells are reflections of this function. It is hoped that experiments will reveal some of the biochemical events controlled by mutant and normal alleles at this locus.

LITERATURE CITED

ADLER, H. I., AND J. C. COPELAND. 1962. Genetic analysis of radiation response in *Escherichia coli*. Genetics **47**:701–712.

ADLER, H. I., AND M. S. ENGEL. 1961. Factors influencing the survival of bacteria after exposure to ionizing radiation. J. Cellular Comp. Physiol. Suppl. **58**:95–105.

DEERING, R. A. 1958. Studies on division inhibition and filament formation of *Escherichia coli* by ultraviolet light. J. Bacteriol. **76**:123–130.

ENGEL, M. S., AND H. I. ADLER. 1961. Catalase activity, sensitivity to hydrogen peroxide and radiation response in the genus *Escherichia*. Radiation Res. **15**:269–275.

GRULA, E. A., AND M. M. GRULA. 1962. Cell division in a species of *Erwinia*. III. Reversal of inhibition of cell division caused by D-amino acids, penicillin, and ultraviolet light. J. Bacteriol. **83**:981–988.

HOWARD-FLANDERS, P., R. P. BOYCE, E. SIMSON, AND L. THERIOT. 1962. A genetic locus in *E. coli* K-12 that controls the reactivation of UV-photoproducts associated with thymine in DNA. Proc. Natl. Acad. Sci. U.S. **48**:2109–2115.

MORSE, M. L., AND C. E. CARTER. 1949. The synthesis of nucleic acids in cultures of Escherichia coli, strains B and B/r. J. Bacteriol. **58**:317–326.

RÖRSCH, A., A. EDELMAN, AND J. A. COHEN. 1963. The gene-controlled radiation sensitivity in *Escherichia coli*. Biochim. Biophys. Acta **68**:263–270.

SHERMAN, J. M., AND W. R. ALBUS. 1923. Physiological youth in bacteria. J. Bacteriol. **8**:127–139.

STAPLETON, G. E. 1955. Variations in the sensitivity of *Escherichia coli* to ionizing radiations during the growth cycle. J. Bacteriol. **70**:357–362.

STAPLETON, G. E., AND M. S. ENGEL. 1960. Cultural conditions as determinants of sensitivity of *Escherichia coli* to damaging agents. J. Bacteriol. **80**:544–551.

VOLKIN, E., AND W. E. COHN. 1954. Estimation of nucleic acids. Methods Biochem. Analy. **1**:287–305.