EFFECTS OF NEAR-ULTRAVIOLET IRRADIATION ON GROWTH AND OXIDATIVE METABOLISM OF BACTERIA

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ABSTRACT

KASHKET, E. R. (Harvard Medical School, Boston, Mass.) AND A. F. BRODIE. Effects of near-ultraviolet irradiation on growth and oxidative metabolism of bacteria. J. Bacteriol. **83**:1094–1100. 1962.—The effects of irradiation with near-ultraviolet light (360 mμ) have been studied with *Escherichia coli* W and a strain of *Pseudomonas aeruginosa*. The growth of the aerobe *P. aeruginosa* was inhibited by light on minimal salts media containing succinate, glutamate, or glucose as sole carbon sources. The facultative anaerobe *E. coli* was capable of growth under irradiation on a fermentable carbon source, such as glucose, but with a smaller yield of cells on limiting substrate, as compared to unirradiated control cultures. The rate of growth of aerobic irradiated cells on glucose was equal to that of anaerobic growth on that carbon source, and there was a greater accumulation of end products of glucose catabolism aerobically in the light as compared to dark controls. When irradiated in media containing carbon sources from which energy was obtainable only by oxidative phosphorylation, such as succinate or malate, *E. coli* cells were still capable of oxidizing these substrates but could not grow on them. This bacteriostatic effect of 360-mμ light could be reversed by the addition of glucose, which resulted in the growth of irradiated cells. Visible (400 to 600 mμ) light was found to have no effect. Irradiated *E. coli* cells in succinate were found to contain no naphtho- or benzoquinones, compounds which are more sensitive to 360-mμ irradiation than other components of the respiratory chain. It is suggested that the effect of 360-mμ light on whole cells is the destruction of light-sensitive components, such as the benzoquinone Q_8 and naphthoquinone K_2C_{45} of *E. coli* W, which are essential for obtaining energy from oxidative metabolism.

Quinones, found in a great variety of organisms (Lester and Crane, 1959; Page et al., 1960), have been established as participating in the oxidative metabolism of bacteria (Brodie and Ballantine, 1960), mammals (Morton, 1960; Crane, 1960; Green, 1960; Martius, 1960; Redfearn, 1960), and plants (Bishop, 1960; Krogmann, 1961). These compounds can be destroyed in bacterial extracts by irradiation with light at 360 mμ (Brodie, Weber, and Gray, 1957) without affecting the structural integrity of enzyme complexes which are essential for coupling phosphorylation to oxidation.

Treatment of intact bacteria with light at this wave length should result in lesions in oxidative metabolism and should be reflected by alterations in the pathways of energy metabolism. Thus, growth of strictly aerobic organisms should be inhibited by irradiation. Facultative organisms should be unable to grow in light in media with carbon sources from which energy is obtainable only by oxidation, but they should grow on fermentable substrates such as glucose. The unavailability of energy from oxidative metabolism during irradiation should alter the rate of aerobic growth on glucose, the yield of cells on limiting quantities of this substrate, and the end products of glucose catabolism. In addition, when cells are irradiated in media with succinate or malate, they should exhibit alterations in the oxidation of these compounds. In the present communication, the effects of light at 360 mμ on the growth and metabolism of bacterial cells have been compared under conditions of fermentation and those of oxidative catabolism. Preliminary reports have appeared elsewhere (Brodie, 1959; Kashket and Brodie, 1960).

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MATERIALS AND METHODS

The bacteria used in this study were *Escherichia coli* strain W and a strain of *Pseudomonas aeruginosa* given to us by R. Leavitt. The bacteria were grown in a liquid medium to exponential phase, harvested, washed, and samples transferred to 250-ml Erlenmeyer flasks with side arms containing 20 ml of fresh medium of the same composition. The growth of the cultures was measured at intervals during aerobic incubation at 37 C by following the increase in optical density in a Klett-Summerson colorimeter (no. 42 filter). Dry weights of *E. coli* cells were calculated from optical density values, with reference to a standard curve.

A minimal salts medium (Moyed, 1960) was used for the growth of *E. coli* cells with 0.5% glucose, 0.5% succinate, or 0.5% malate as the sole carbon and energy source. The *P. aeruginosa* cells were grown on a medium consisting of 0.2% K_2HPO_4, 1% $(NH_4)_2SO_4$, 0.08% $MgSO_4 \cdot 7H_2O$, 0.04% NaCl, 0.004% $FeSO_4 \cdot 7H_2O$, and 0.01% $MnSO_4 \cdot 4H_2O$, supplemented with 1% sodium glutamate, 0.5% glucose, or 0.5% succinate. Viability counts were determined by plating on agar plates consisting of Bacto-Tryptone Glucose Extract Agar (Difco).

The cultures were irradiated by placing an 8-w black Raymaster type B lamp (maximal emission, 360 mμ) in a standard shaking apparatus. Each lamp was used for not more than 20 hr, as its effect was found to diminish after longer use. The flasks were clamped at a distance of 2 cm from the lamp, and the unirradiated control flasks were wrapped in aluminum foil. The shaker was placed in an incubator (37 C), and a fan was used to keep the temperature constant. Visible light was provided by a General Electric Daylight 8-w lamp in the same position. Irradiation of nongrowing whole cells was carried out by a 100-w Uvilux (Burton Manufacturing Co., Santa Monica, Calif.) black light (bulb no. BU-104; maximal emission, 366 mμ).

Glucose utilization was measured enzymatically by the Gluostat (Worthington Biochemical Corp., Freehold, N.J.) method, and lactate accumulation by the method of Barker and Summerson (1941). The oxidation of succinate and malate by the *E. coli* cells was measured by conventional manometric techniques in a Warburg apparatus at 30 C. Benzoquinone was extracted from the cells and its concentration measured spectrophotometrically (Pumphrey and Redfearn, 1960). Flavin concentrations were measured by the method of Burch, Bessey, and Lowry (1948).

RESULTS

E. coli. The facultative organism chosen to test the effects of light on growth was *E. coli* W. Cells previously grown aerobically on glucose were transferred to a glucose-containing minimal medium and aerated in the presence and absence of light at 360 mμ. Cells grew faster in the dark than when irradiated during incubation (Fig. 1). Viable counts confirmed the increase in the number of cells. With succinate as the carbon source, however, succinate-adapted *E. coli* cells grew in the dark after a short lag, but failed entirely to grow under irradiation. When glucose was added to such an arrested culture, either at 4 or 24 hr after cessation of growth, growth resumed and usually exhibited a rate characteristic of irradiated cells growing on glucose. Further evidence that irradiated cells in a succinate medium are viable was obtained by plate counts (on tryptic digest agar, taken before and after light treatment), which revealed a slight increase in the number of cells undetectable by turbidimetry. Similar cessation of growth was observed when *E. coli* cells were irradiated in a malate medium. Such cultures also resumed growth in the light when glucose was added.

Irradiation of *E. coli* cells in succinate with visible light (400 to 600 mμ) did not affect the growth of these organisms nor did it have any effect on cells previously irradiated at 360 mμ in the same medium.

Although *E. coli* cells remained viable after light treatment (360 mμ) in succinate medium, the effect of light on succinate utilization for growth appears to be irreversible over the period measured. No growth was observed when irradiated *E. coli* cells in succinate medium were placed in the dark (Fig. 2). However, when such cells were allowed to double by the addition of glucose, while still being irradiated, growth on succinate in the dark resumed, but only after an extended lag period (Fig. 3). Control unirradiated *E. coli* cells, after growth in glucose, exhibited a lag required for adaptation to succinate. The increased lag of irradiated cells suggests that resynthesis of light-sensitive

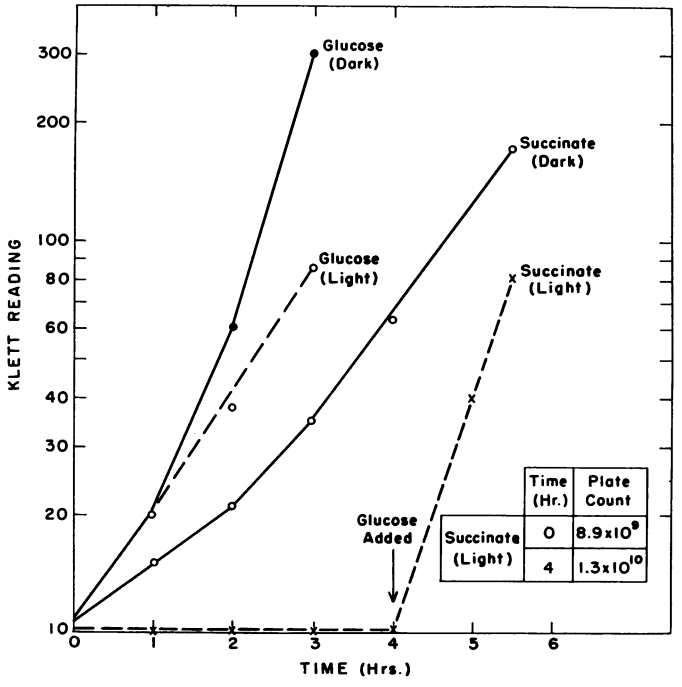

FIG. 1. *Comparison of the growth of irradiated and unirradiated Escherichia coli W in glucose and succinate minimal media.*

components essential for growth on succinate can take place if the cells are provided with energy and possible precursors derived from glucose. The length of the lag period was dependent upon the duration of prior irradiation of the cells in succinate (Table 1).

P. aeruginosa. Since light at 360 mμ affects oxidative metabolism, a strict aerobe was incubated in the presence and absence of light. *P. aeruginosa,* which is incapable of fermenting glucose (Lockwood, Tabenkin, and Ward, 1941), failed to grow during irradiation in glucose (Fig. 4), succinate, or glutamate-containing media. These results were anticipated, since these organisms can obtain energy only from oxidative metabolism.

Biochemical studies. Since these results indicated that the utilization of the aerobic pathways for energy metabolism could be blocked effectively by light without interfering with anaerobic catabolism, the fermentative and oxidative pathways after light exposure were examined.

With a limiting amount of succinate as substrate, *E. coli* cells grown in a succinate-containing medium in the dark were able to oxidize about 65% of the available substrate and to

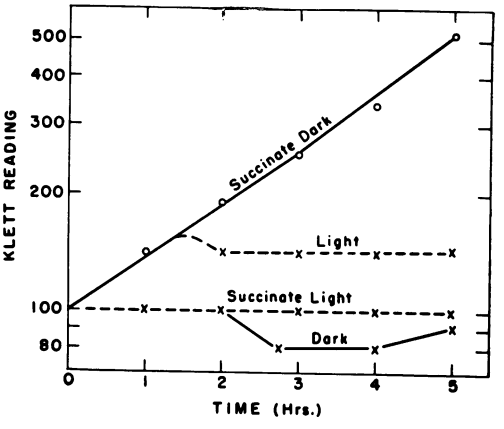

FIG. 2. *Effect of reversal of light and dark conditions. Escherichia coli W, previously grown in succinate minimal medium, was transferred into similar medium. Two cultures were irradiated and two wrapped in aluminum foil. After 2 hr, one of the irradiated flasks was wrapped in foil and one of the unirradiated cultures was exposed to light at 360 mμ.*

assimilate the remainder (Fig. 5). The addition of 2,4-dinitrophenol (DNP) inhibited assimilation, thus increasing oxidation almost to the theoretical values. Irradiated cells, although

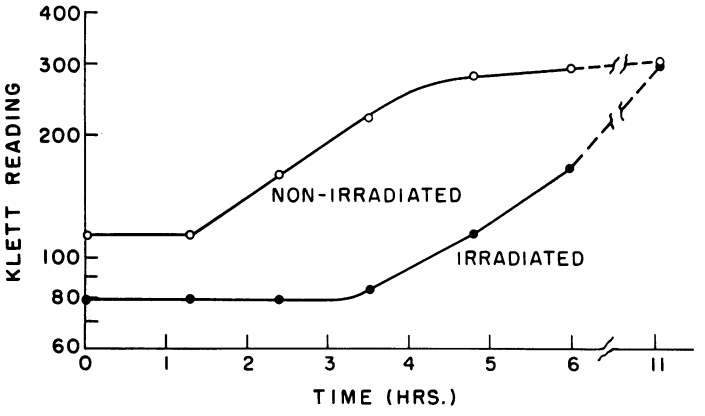

FIG. 3. *Reconstitution of ability of irradiated cells of Escherichia coli W to grow in succinate. A culture of E. coli was irradiated in succinate medium, as in Fig. 1. After cessation of growth (210 min), glucose was added and the cells allowed to double, still in the light. The cells were harvested, washed twice with distilled water, and resuspended in succinate medium in the dark. An unirradiated control culture was treated similarly.*

unable to use succinate for growth, were still capable of oxidizing this substrate, but more slowly. This oxidation differed from that of cells grown in the dark in that assimilation did not occur, since oxidation was unaffected by the presence of DNP. The oxidation of malate by cells irradiated in a malate-containing medium was similarly affected.

To determine whether irradiation of *E. coli* in glucose-containing media alters the pattern of energy metabolism, glucose utilization and lactate production by irradiated and unirradiated cells were measured. Glucose consumption was nearly equal in irradiated (263 μmoles/mg dry wt) and unirradiated cells (260 μmoles/mg dry wt), both cultures consuming 80% of the initial glucose (Table 2). However, the irradiated bacteria accumulated more lactate than the unirradiated control cells (Table 2). When light-treated cultures had reached the stationary phase, 7.2% of the supplied glucose was recovered as lactate, as compared to 2.5% with unirradiated controls. The extent of accumulation of lactate did not approach that of anaerobically growing *E. coli*, where up to 44% of supplied glucose is recovered as lactate (Stephenson, 1950). This may be a reflection of the capacity of irradiated cells to oxidize end products of glucose metabolism further.

With limiting concentrations of glucose, light-treated *E. coli* cells grew aerobically to a lower density than unirradiated aerobic control cultures. The yield of irradiated cells (dry wt/unit volume of culture) was 14.5% less than dark controls at glucose concentrations of 0.03 and 0.1%. The rate of growth of irradiated aerobic cells was found to equal that of anaerobically growing cells (doubling time: 94 min), both rates being slower than that of unirradiated aerobically growing cultures (doubling time: 63 min). These data support the conclusion that irradiated *E. coli* cells cannot obtain energy from oxidative metabolism, whether in glucose, succinate, or malate-containing media, and that in glucose media they are capable of growth because energy is available from fermentation.

E. coli W cells contain both a benzoquinone, Q_8, and a naphthoquinone, K_2C_{45} (Lester and Crane, 1959; Kashket and Brodie, 1960; Brodie,

TABLE 1. *Adaptation of Escherichia coli W to growth on succinate after light treatment (360 mμ)**

Irradiation	Lag†
min	*min*
100	45
210	120
320	160

* The conditions were the same as in Fig. 3. The duration of irradiation of *E. coli* W cells in succinate medium was varied. After light treatment, the cells were grown for one generation on glucose in the light, and then transferred to succinate-containing medium in the dark. The lag in resumption of growth in succinate was measured.

† Corrected for lag of unirradiated controls.

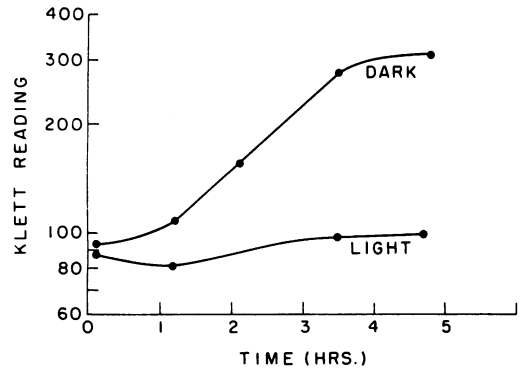

FIG. 4. *Effect of light at 360 mμ on the growth of Pseudomonas aeruginosa in minimal media containing glucose as the sole carbon and energy source.*

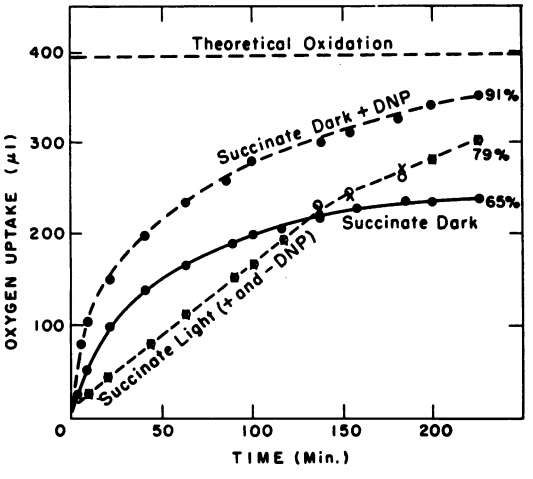

FIG. 5. *Oxidation of succinate by light and dark grown cells. Cells of Escherichia coli W were irradiated and grown in succinate minimal medium for 5 hr, as in Fig. 1. After harvesting and washing twice with distilled water, the cells were tested at 30 C for ability to oxidize succinate (5 μmoles) in the presence and absence of 1 × 10^{-4}M DNP. Each vessel contained 3.9 × 10_3 mg (dry wt) of bacteria.*

1961), in concentrations compatible with function in oxidative metabolism. The naphthoquinone is more sensitive to light at 360 mμ than the benzoquinone, both in pure solution (Brodie, 1962) and in cell-free extracts of *E. coli* (Kashket and Brodie, 1961). Cultures of *E. coli* in succinate minimal medium, used in growth studies (Fig. 1), on lipid extraction yielded solutions too dilute for colorimetric determination of quinones; however, ultraviolet-absorption spectra indicated the presence of the benzoquinone Q_8 in unirradiated cells and its absence in light-treated cultures. The effect of 360-mμ light on quinones and flavins was observed during irradiation of a large number of cells grown in malate and harvested during exponential growth (Table 3). Under these conditions, the benzoquinone content was diminished by 83.4% while the flavin adenine dinucleotide (FAD) concen-

TABLE 2. *Effect of irradiation (360 mμ) on glucose utilization and lactate production by Escherichia coli* W*

Time	Irradiated			Unirradiated		
	Dry weight	Glucose consumption	Lactate accumulation	Dry weight	Glucose consumption	Lactate accumulation
min	*μg*	*μmoles*	*μmoles*	*μg*	*μmoles*	*μmoles*
0	1.9	0	0	1.9	0	0
210	34.3	4.1	0	67.4	11.9	0.3
225	—	—	—	83.8	20.1	0.5
315	76.1	15.8	0.5	104.4	27.5	0.7
375	96.9	25.2	1.8	—	—	—

* *E. coli* W cells were grown aerobically in 10 ml of minimal medium (0.3% glucose) as in Fig. 1. At intervals the optical densities of the cultures were measured and 1-ml samples removed. These were centrifuged to remove the cells, and the supernatants fluids were analyzed for glucose and lactate.

TABLE 3. *Effect of 360-mμ irradiation on the benzoquinone and flavin content of Escherichia coli W**

Irradiation (360 mμ)	Respiratory component (mμmoles/mg dry wt)		
	Benzoquinone	FAD	FMN
min			
0	84.2	1.83	9.69
45	59.8	2.34	8.73
60	36.4	2.15	8.55
75	14.0	2.19	7.31
% Loss,	83.4	0	24.6

* *E. coli* W cells were grown in malate to exponential phase, harvested, washed, irradiated while suspended in 20 ml of water (0.89 mg dry wt/ml) and mixed in an open 400-ml beaker in an ice bath. A 100-w Uvilux black light (maximal emission: 366 mμ) was placed 16 cm from the cell suspension. At various intervals, samples of cells were removed for benzoquinone, FMN, and FAD analysis. The naphthoquinone concentration was too low for measurement.

tration was not lowered and riboflavin 5′-phosphate (FMN) diminished by 24.6%. The cytochrome pigments are not affected by exposure to light at this wave length (Weber, Brodie, and Merselis, 1958). Thus, the quinones appear to be the most light-sensitive respiratory component examined.

DISCUSSION

Oxidative pathways of energy metabolism include components sensitive to irradiation at 360 mμ. This is demonstrated by the absence of growth of the aerobe *P. aeruginosa* under irradiation in succinate, glutamate, or glucose minimal salts media. The facultative bacterium *E. coli* W failed to grow when irradiated under conditions in which energy was obtained only by oxidative phosphorylation, while growth took place in the presence of a fermentable carbon source. The alteration in the biochemical properties of light-treated *E. coli* cells, such as the uncoupled nature of succinate oxidation, and the slowing of growth on glucose to the rate characteristic of anaerobiosis, indicate the vital role of light-sensitive components in oxidative metabolism.

The effect of irradiation with near-ultraviolet (360 mμ) light radically differed from that of far-ultraviolet (253.7 mμ) light (Hollaender, 1943). In the present studies, the former produced no lethal effects on the facultative anaerobe *E. coli* W, the cessation of growth under irradiation on succinate or malate being bacteriostatic. Far-ultraviolet irradiation is bactericidal (Gates, 1929) and mutagenic (Henri, 1914), producing effects some of which can be partially reversed by irradiation with visible light (Kelner, 1949; Zelle and Hollaender, 1955). It is interesting that 360-mμ light was found most effective in reversing far-ultraviolet mutagenesis and killing (Newcombe and Whitehead, 1951). As their irradiation with 365-mμ light was effective in as short a time as 2 min, it is difficult to interpret their results in the light of the present findings with 360-mμ irradiated *E. coli* cells. Such a short time of exposure to light at 360 mμ would not be sufficient to affect the quinones. Visible light (400 to 600 mμ) was found to have no effect on 360-mμ irradiated *E. coli* W.

Likely candidates for light-sensitive components in the oxidative metabolism of bacteria are the quinones and the flavins. Irradiation of *E. coli* extracts with light at 360 mμ inhibits both phosphorylation and oxidation, with a concomitant loss of quinones from the preparation (Kashket and Brodie, 1961). Lipid extraction of cell-free extracts, which removes the quinones, also results in a loss of oxidative activity. The absence of quinones in nongrowing *E. coli* cells irradiated in succinate media and their presence in growing unirradiated control cultures, together with the greater sensitivity of quinones to irradiation as compared to other components of the respiratory chain, suggest that inhibition of growth of whole cells by 360-mμ light is due to the destruction of quinones. Thus, quinones seem to play a role in oxidative metabolism in growing whole cells, as well as functioning catalytically in cell-free extracts.

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