THE EFFECT OF X RADIATION ON THE RESPIRATION OF ESCHERICHIA COLI[1]

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Few studies have been conducted on the effects of X irradiation on microbial respiration. Barron and Gasvoda (*personal communication*) have shown that when resting suspensions of *Corynebacterium creatinovorans* were irradiated with 6,000 r, the endogenous respiration remained unaffected. However, the oxidation of acetate was 25 per cent inhibited, and the oxidation of aspartate, 6 per cent inhibited. Brandt *et al.* (1951) have reported that an X-ray dose of 4,850 r had little effect on the respiration of yeast cells although 90 per cent of the cells were unable to form visible colonies on plating. Sherman and Chase (1949) observed an inhibition of fermentation in yeast cells that had been exposed to 90,000 r.

We have found in resting cell studies that X rays inhibit the respiratory mechanism of *Escherichia coli*. The degree of inhibition varies with the substrate oxidized, dosage given, temperature at which the respiration studies are conducted, and strain of the organism used.

EXPERIMENTAL METHODS

Escherichia coli, strain B/r, was used in all experiments with the exception noted. Cultures were grown for 18 hours under constant aeration at 37 C in 0.8 per cent nutrient broth (Difco). Suspensions, harvested by centrifugation and washed in M/15 phosphate buffer at pH 6.8, were brought to original volume in the phosphate buffer. Final suspensions prepared in this manner contained approximately 2×10^9 cells per ml. Then samples of the suspensions were exposed to X rays (60,000 r in all cases except for dose studies) at ice bath temperatures as described by Stapleton *et al.* (1952*a*). Platings of both the exposed and unexposed suspensions were made to determine the number of viable organisms present. Respiratory activity was determined by measuring oxygen uptake at 37 C, using conventional manometric techniques. The main compartment of each cup contained 0.5 ml of the cell suspension plus 1.0 ml of M/15 phosphate buffer at pH 6.8. Two-tenths of a milliliter of 20 per cent KOH was placed in the center well. The liquid content of each cup was brought to a final volume of 3.0 ml with distilled water. The substrate (20 μM unless otherwise specified) was tipped in from the side arm after equilibration of the contents of the cup. The equilibration period required from 10 to 15 minutes.

Cells grown and treated in the manner described had little or no endogenous respiration either before or after irradiation.

EXPERIMENTAL RESULTS

The data in figure 1 show that a suspension of cells exposed to 60,000 r respires initially at the same rate as the unexposed control cells. The duration of the normal activity period varies with the substrate, being longer on pyruvate or succinate than on glucose. Following this period of normal activity, there is a rapid decrease in the respiratory rate of the exposed cells as compared to the rate exhibited by the unexposed cells. The number of cells in the exposed suspension that are capable of forming visible colonies is about 0.05 per cent of the number found before irradiation. Thus, the respiratory rate of the exposed cells is not consistent with the number of viable cells determined from plate counts. The observed normal oxygen consumption rate must be due, at least initially, to a major portion, if not all, of the exposed cells partaking in the oxidation of substrate. We have not been able to determine whether the drop in respiratory rate that follows the normal activity period is a reflection of a gradual decrease in individual cell activity or whether it is a result of complete cessation of the activity of an increasing number of the affected cells. The cause of the increment in pyruvate oxidative rate of the control cells at 75 minutes is not known. This increment in

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oxidative rate on pyruvate does not take place in the exposed cells. It is suggested that new enzyme formation has taken place in the control cells but not in the exposed cells. One effect of an X-ray dose of 60,000 r on enzyme formation is the inhibition of the capacity of *E. coli* (Texas strain) to form the adaptive enzyme, formic hydrogenlyase (Billen and Lichstein, 1952).

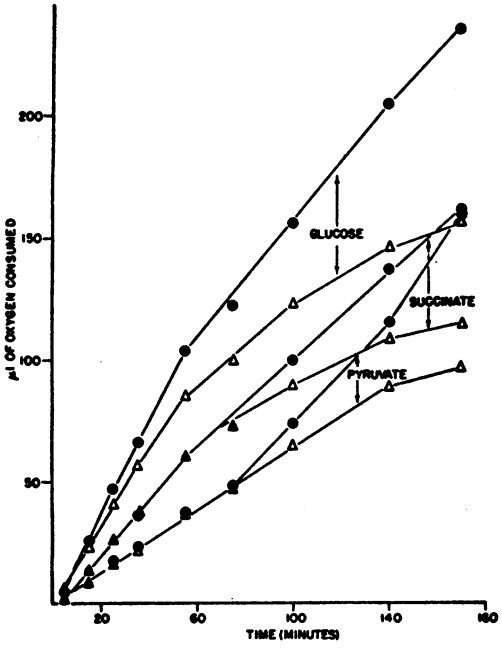

Figure 1. The respiratory activity of X irradiated *Escherichia coli*, strain B/r, on several substrates. The control cups contained 20×10^8 and the experimentals 20×10^4 colony forming organisms. ● = Controls; △ = irradiated cells.

Similar experiments, utilizing *E. coli* (Texas strain), were conducted to ascertain whether the results obtained with the B/r strain on glucose and pyruvate oxidation were a general response of all X irradiated *E. coli*. With irradiated *E. coli* (Texas strain) an immediate inhibition of pyruvate oxidation was noted while glucose oxidation was unaffected for approximately 80 minutes (figure 2). Thus, in the two strains studied, a marked difference in X radiation damage on glucose and pyruvate oxidation is noted. The data suggest a glucose oxidizing system in the Texas strain in which pyruvate formation plays no role. If glucose was being oxidized through a series of steps which involved pyruvate as an intermediate, then one would expect an immediate inhibition as found with pyruvate oxidation. Further studies with X irradiated cells may prove the usefulness of ionizing radiations as a tool in determining common or divergent pathways in the utilization of two or more substrates.

A study of the relationship of X-ray dose to respiratory activity of exposed cells on glucose indicated that the duration of normal respiratory activity was relatively unaffected by increasing the dose from 5,000 to 90,000 r (figure 3). However, once the inhibition manifested itself, the decrease in rate was more pronounced in those cells exposed to the higher doses.

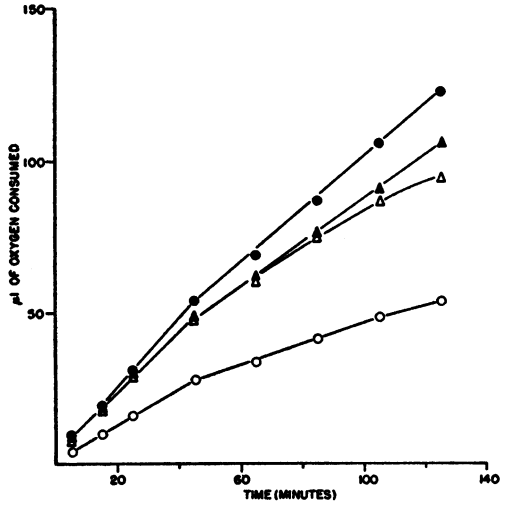

Figure 2. The respiratory activity of X irradiated *Escherichia coli* (Texas strain) on glucose and pyruvic acid. The control cups contained 12×10^8 and the experimentals 46×10^4 colony forming organisms. ● = Pyruvate-control; ○ pyruvate-irradiated; ▲ = glucose-control; △ = glucose-irradiated.

A comparison of the respiratory quotient on a limited glucose concentration (1.0 μM) revealed a value of 1.0 for both the exposed and unexposed cells although the former required a longer period of time to complete the oxidation of the added substrate. The total oxygen consumed for both types of cells was 74 per cent of the calculated amount required for complete oxidation of the glucose to CO_2. Although the data are limited, it would appear that the oxidative pathway is not altered by X rays; however, the rate of oxidation is somehow affected.

That the enzyme activity of the exposed cells

was adversely affected by holding in phosphate buffer at 37 C is shown in figure 4. Incubating the cells for 60 minutes in the manometer cups before tipping in glucose decreased the duration of the normal respiratory activity period of the irradiated cells. No effect was noted on the rate of oxygen uptake of the control cells. It was found also that exposed cells stored at ice bath temperature (1 C) for 3 hours after X-ray treatment

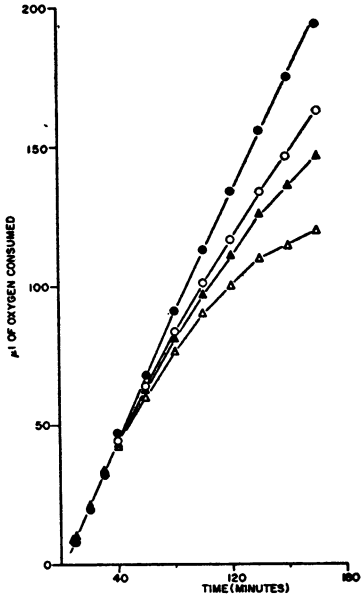

Figure 3. The effect of X-ray dose on the respiratory activity of *Escherichia coli*, strain B/r. Colony forming organisms per cup were as follows: control cup, 11×10^8; cup A, 68×10^7; cup B, 24×10^7; cup C, 23×10^6. The substrate added was glucose and the bath temperature was 32 C. ● = Control; ○ = 5,000 r (A); ▲ = 15,000 r (B); △ = 90,000 r (C).

had the same respiratory activity as cells studied immediately after exposure.

The influence of temperature on the observed accelerated decay in respiratory activity of the exposed cells in the presence of substrate was investigated next. A definite retardation of the X-ray induced inhibition of respiration was found at 26 C when compared with that observed at 37 C (figure 5). Both the duration of normal respiratory activity and the oxygen uptake during this period surpassed that of the cells which were held at 37 C. It was noted also that, at the end of 4 hours, the total oxygen uptake by exposed cells held at 26 C was greater than the total uptake observed during the same period by exposed cells held at 37 C. Apparently then,

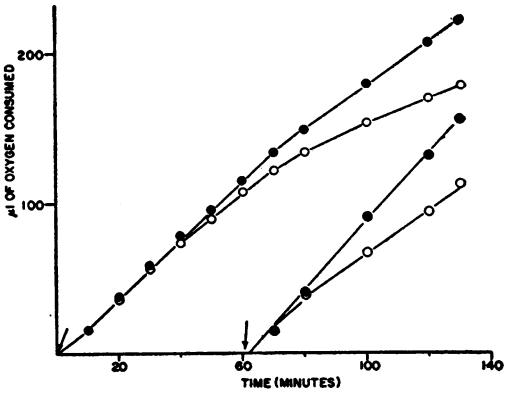

Figure 4. The effect of incubation at 37 C following X irradiation, on the respiratory activity of *Escherichia coli*, strain B/r. The control cups contained 20×10^8 and the experimentals 22×10^4 colony forming organisms. The arrows indicate time of glucose addition. ● = Control cells; ○ = irradiated cells.

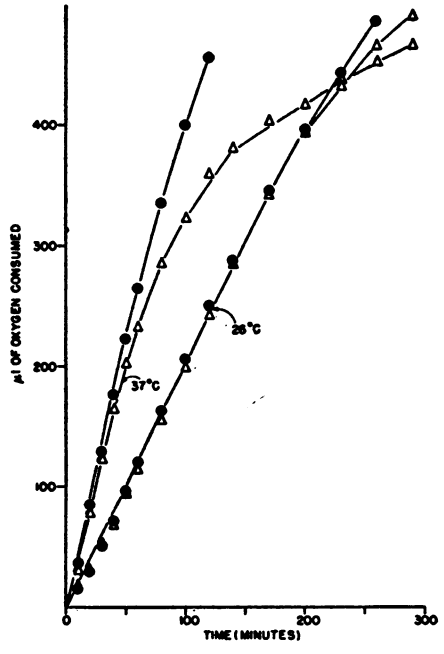

Figure 5. The influence of temperature on X-ray damage to bacterial respiration. The control cups contained 54×10^8 and the experimentals 56×10^4 colony forming organisms. ● = Controls; △ = irradiated cells.

the higher temperature increases whatever damage the X rays may have done directly or indirectly to the respiratory system.

DISCUSSION

Comparative studies of normal and irradiated cells to ascertain X-ray damage, using criteria other than viability as assayed by plate counts, can lead to conclusions conflicting with those reached on the basis of viability. We have presented evidence that an essentially dead suspension of *E. coli* (0.05 per cent survivors) based on plate counts may respire initially at a normal rate. Rahn and Barnes (1933) observed relatively little apparent killing in yeast cells exposed to X rays when the ability to stain with methylene blue was used as a criterion of death. They found that at a given exposure, 21.4 per cent survived, based on colony forming capacity, while 95.8 per cent survived, based on dead cells staining with methylene blue. We have observed (*unpublished data of the authors*) that when cells, previously exposed to 60,000 r, are inoculated in sufficient numbers into nutrient broth and incubated at 37 C, there is a limited increase in growth based on turbidity. This turbidity increase can be accounted for only by assuming that most, if not all, of the cells are active initially. Thus, it would appear that, immediately following X irradiation, the exposed cells are quite similar to control cells, at least in the several characteristics studied. After the enzyme systems have been induced to work by the addition of substrate, or if the cells are exposed to higher than ice bath temperatures, an abnormal deterioration in enzyme activity may take place, as found in the respiratory system.

At the present time, any attempted explanation of the initial similarity in oxidative rates between exposed and nonexposed cell suspensions and the subsequent rapid decrease in the rate of the exposed cells must of necessity be pure speculation. Giese (1941) observed a similar response in the respiration of *Achromobacter fischeri* that had been exposed to ultraviolet radiation. He found that, at low doses of ultraviolet, the cells respired for a considerable length of time at a rate equal to that of the control, and that the duration of this period of normal activity decreased as the dosage was increased.

There are three interpretations that warrant consideration, the first two being somewhat similar to those postulated by Giese (1941) in his discussion on the mode of action of ultraviolet radiation on bacterial respiration. It may be that normal, resting cells in the absence of exogenous nitrogen do possess the ability to replace by synthesis enzymes that deteriorate either through use in the presence of substrate or by incubation at temperatures causing inactivation of enzymes. This replacement may be from some pre-enzyme state of reserve protein. If the cell, by exposure to X radiation, loses the faculty of replacing inactivated enzyme molecules, then one would expect the respiratory rate to decrease at a more rapid pace after a period of time. X-ray inhibition of the capacity of *E. coli* to form the enzyme, formic hydrogenlyase, has been demonstrated (Billen and Lichstein, 1952). It is possible also that a reserve of respiratory enzymes exists which could replace that fraction of enzymes inactivated during irradiation. Thus, the period of normal activity of the exposed cells would be directly dependent on the total enzyme content of the cells before exposure, and the period of normal activity should decrease with increasing dose of X rays. However, the data (figure 3) indicate that increasing dose has relatively little effect on the length of the period of normal respiratory activity. A third possibility is that incubation of the exposed cells in the temperature range over which enzymes are active initiates a chemical or physical disruption of the organization of the enzymatic systems concerned with the respiratory activity of the cell. Thus, the observed normal period of respiration followed by the rapid decrease in oxygen consumption would reflect secondary damage initiated by the effects of the X rays on some other loci.

The explanation of the extended duration of normal respiratory activity by exposed suspensions of *E. coli*, strain B/r, on pyruvate and succinate as compared to that on glucose is obscure at present. It is possible that different oxidative pathways exist for these metabolites and that the glucose oxidizing system is more sensitive than the pyruvate or succinate systems. If glucose is oxidized through a system involving pyruvate as an intermediate (as in glycolysis), then the greater sensitivity of the glucose oxidizing system could be a reflection of the radiation effect on the additional enzymes of the glycolytic system required for glucose dissimilation to pyruvate. The reasoning here is

that the greater the number of enzymatic steps involved in a system the greater will be the probability of X rays affecting that system. It has been demonstrated previously that the breakdown of formate by formic hydrogenlyase, supposedly a one step system, is unaffected by exposure to 90,000 r of the cells containing this enzyme (Billen and Lichstein, 1952).

The delay and modification of X radiation effects on respiration by low temperatures are of particular interest because of their implications. The observation that the total activity of the oxidative system in exposed cells is greater within a given time at 26 C than at 37 C is suggestive of the existence of an abnormally heat-sensitive respiratory system in these cells. Giese and Heath (1948) reported that sublethal dosage of soft X rays increased the sensitivity of *Paramecium caudatum* to heat. We have observed also that cells held in phosphate buffer after irradiation show a marked drop in number of viable cells if the incubating temperature is 37 C as compared to a less pronounced decrease at a temperature of 25 C and below (*unpublished data of the authors*). The unexposed cells were relatively little affected by such treatment. It is also significant that the number of viable survivors of X irradiated *E. coli*, strain B/r, suspensions may be increased by incubation in the proper medium at temperatures below 30 C (Stapleton *et al.*, 1952*b*) with the maximum number of survivors being obtained at 18 C. Thus, it becomes evident that the effect of X rays on the respiratory system may be altered by varying the conditions which affect the metabolic functions of the cell.

SUMMARY

The effect of ionizing radiations, in the form of X rays, on the respiratory system of *Escherichia coli* has been studied. It was observed that there is an initial period of normal respiration by *E. coli*, strain B/r, following exposure to an X-ray dose that reduced the number of colony forming cells by more than 99.9 per cent. This period of normal activity is substrate dependent since it was found to be of longer duration on pyruvate and succinate than on glucose. A period of normal respiratory activity on glucose, in contrast to an immediate inhibition of oxidative activity on pyruvate, was observed with *E. coli* (Texas strain). On the basis of the data presented, it is suggested that ionizing radiations may prove to be a useful tool in determining common or divergent pathways in the utilization of two or more substrates by a microorganism.

The duration of the normal respiration on glucose shown by exposed cells was relatively little affected by increasing dose (5,000 to 90,000 r). It was found also that the inhibition of the respiratory activity of the exposed cells was more pronounced if respiration was followed at 37 C than if studied at 26 C. If the cells were incubated at 37 C for 60 minutes before adding substrate, the duration of the normal respiratory period was decreased.

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