THE EFFECT OF X RADIATION ON THE ADAPTIVE FORMATION OF FORMIC HYDROGENLYASE IN ESCHERICHIA COLI[1]

DANIEL BILLEN AND HERMAN C. LICHSTEIN[2]

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Received for publication October 1, 1951

Brandt *et al.* (1951) have reported that ultraviolet radiation from a sterilamp (36,000 ergs per mm^2) of *Saccharomyces cerevisiae* inhibited completely the adaptive formation of galactozymase. In contrast, a dosage of 4,850 r of X-rays had no noticeable effect on adaptation to this enzyme, in spite of the fact that it prevented division of 90 per cent of the cells.

During studies on the mechanism of X radiation effects on microorganisms conducted in this laboratory, the adaptive formation of formic hydrogenlyase in resting cell suspensions of *Escherichia coli* was investigated. The present paper reports on the lability of this system to X radiation.

METHODS

Stationary cultures of *Escherichia coli*, strain Texas, were employed in these studies utilizing the media and techniques described by Billen and Lichstein (1951). X irradiation of the cell suspensions was conducted at constant temperature in an ice bath according to the procedures reported by Anderson (1951). Plate counts of appropriate dilutions were made in all experiments utilizing nutrient agar, and 18 hours' incubation at 37 C.

The adaptive formation of formic hydrogenlyase was followed manometrically at 37 C using conventional Warburg techniques. The main compartment of the Warburg cup contained the washed suspension of *E. coli*, harvested from a glucose-mineral salts medium (Billen and Lichstein, 1951), suspended in M/20 phosphate buffer (pH 6) to which were added 0.2 ml of 0.001 M magnesium sulfate and 20 mg of sodium chloride. The side arm contained sodium formate (1.36 mg) and glutamate (12 mg), while 0.2 ml of 20 per cent potassium hydroxide was added to the inner well. The total volume per flask was 2 ml. After temperature equilibration, the contents of the side arm were tipped and hydrogen evolution measured.

RESULTS

The data of a typical experiment are graphically presented in figure 1. It may be seen that the control cells produce the formic hydrogenlyase enzyme rather rapidly with a definite increased rate after approximately 80 minutes. X irradi-

[1] Work performed under Contract No. W-7405-Eng-26 for the Atomic Energy Commission.

[2] On leave from the Department of Bacteriology and Immunology, University of Minnesota, Minneapolis.

ation at several doses produces profound effects on the ability of these cells to form this enzyme. The lowest dose employed (15,000 r) resulted in cells capable of synthesizing enzyme at a rate distinctly lower than the control cells, while an exposure of 30,000 r reduced the rate to an even greater extent. Inspection of the results at 60,000 and 90,000 r shows that these dosages inhibited completely the ability of the cells to produce formic hydrogenlyase. In agreement with previous results (Billen and Lichstein, 1951) it was found that the control cells produced no formic hydrogenlyase unless glutamate or another suitable amino acid was added to the Warburg vessel.

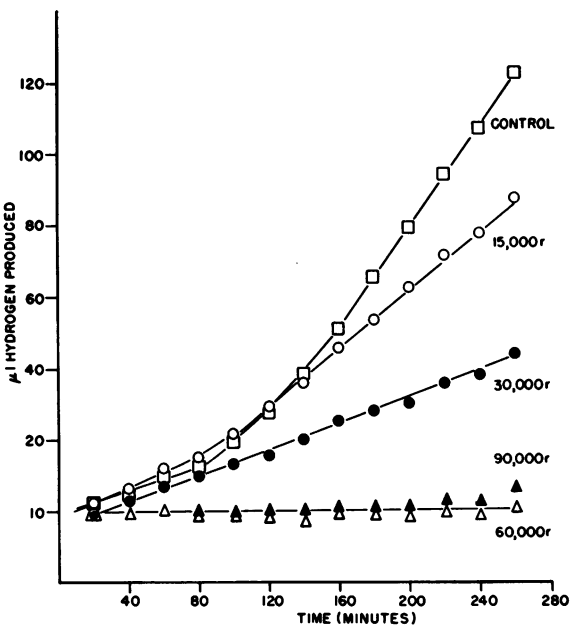

Figure 1. The effect of X radiation on the adaptive formation of formic hydrogenlyase by resting cell suspensions of *Escherichia coli*.

The effect of radiation on this system results primarily in an inability to synthesize enzyme, rather than an effect on preformed enzyme. The irradiation (90,000 r) of cells harvested from the same medium containing acid-hydrolyzed vitamin-free casein (Billen and Lichstein, 1951) had no effect on the existing formic hydrogenlyase. The control cells produced 376 μl hydrogen in two hours and the irradiated cells 387 μl hydrogen during the same period. The rates of hydrogen production were approximately the same for both.

Plate counts in this particular experiment showed the following number of colonies per ml: control, 75×10^8; 15,000 r, 113×10^6; 30,000 r, 221×10^1; 60,000 r, 2×10^2; 90,000 r, less than 10. On the basis of ability to form a visible colony these results show that, in all instances, less than 2 per cent of the irradiated cells were viable. Therefore, it is pertinent to point out that such suspensions were still capable of synthesizing enzyme, and at a rate not consistent with

the number of viable cells as indicated by visible colonies (figure 1, 15,000 and 30,000 r).

ACKNOWLEDGMENT

It is a pleasure to acknowledge the assistance of Mrs. R. B. Grayson.

SUMMARY

The data presented show that X radiation of resting cell suspensions of *Escherichia coli*, in doses of 60,000 r or greater, prevents completely the ability to synthesize formic hydrogenlyase, while having no effect on preformed hydrogenlyase.

REFERENCES

ANDERSON, E. H. 1951 Effect of oxygen on mutation induction by X rays. Proc. Natl. Acad. Sci., **37**, 340–349.

BILLEN, D., AND LICHSTEIN, H. C. 1951 Nutritional requirements for the production of formic hydrogenlyase, formic dehydrogenase, and hydrogenase in *Escherichia coli*. J. Bact., **61**, 515–522.

BRANDT, C. L., FREEMAN, P. J., AND SWENSON, P. A. 1951 The effect of radiations on galactozymase formation in yeast. Science, **113**, 383–384.