THE PROTECTION OF BACTERIA BY PYRUVATE AGAINST RADIATION EFFECTS

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The lethal and mutational effects of radiations are no longer generally regarded to be a consequence solely of the change induced directly in the genetic substance of the organism by the radiant energy. Sufficient evidence is at hand to suggest that the primary change may be in nongenic material to produce some chemical substance or substances which then interact with the genetic mechanism to bring about the biological response. With x-rays the radio-decomposition products of water, which include hydrogen peroxide and HO₂ and OH radicals, may make a large contribution to the observed effect of the irradiation of living organisms. We have shown (Wyss et al., 1947) that a number of the effects of ultraviolet irradiation can be duplicated by peroxides which may be formed in the irradiation process. The dose-response curves obtained with different mutations suggest that genetic changes in bacteria exposed to ultraviolet may be induced indirectly. The presence of intermediate steps between the absorption of the irradiation and the biological response has several important implications: (1) there is a greater possibility of preventing damage from irradiation and alleviating damage already done if these indirect effects are of considerable magnitude, (2) if a series of steps are involved, the elucidation of these may reveal a method by which certain of the biological effects of radiations can be vitiated without modifying others thus resulting in our ability to produce a more specific biological response.

Considerable protection from the killing of bacterial cells by x-radiation results from growing the cells anaerobically in the presence of glucose and by excluding oxygen during the irradiation process (Hollander et al., 1951). The addition of physiological reducing substances such as cysteine and glutathione reduces the lethal action of radiations (Latarjet and Ephrati, 1948). Since pyruvic acid is a normal component in cell metabolism and since it is spontaneously oxidizable by H₂O₂ thus,

\[ \text{CH}_3\text{COOH} + \text{H}_2\text{O}_2 > \text{CH}_3\text{COOH} + \text{CO}_2 + \text{H}_2\text{O}, \]

we have investigated the protection afforded bacteria by this compound against the lethal and mutagenic effects of radiations.

MATERIALS AND METHODS

Carefully washed log phase cultures of *Bacillus anthracis*, *Escherichia coli*, and *Micrococcus pyogenes* var. *aureus* were used. Adequate precautions were employed to control the light reactivation phenomenon (Novick and Szilard, 1949; Kelner, 1949). Nutrient agar (Difco) was used as the plating medium.
both for total counts and for determining mutation to streptomycin resistance. A General Electric Model XRD Type I x-ray instrument was the source of ionizing radiation, and for ultraviolet irradiation a Hanovia, double U, quartz, mercury vapor lamp operating at 20 milliamperes was used. During irradiation the cell suspension was stirred by aeration. The pyruvate was recrystallized as the sodium salt, and the stock solution was adjusted to pH 7.0 before sterilizing by filtration.

![Graph](http://jb.asm.org/)  
*Figure 1. Protection by pyruvate of buffered suspensions of *M. aureus* against exposure to peroxide. A. 0.5 per cent pyruvate present during exposure. B. Control.*

**EXPERIMENTAL RESULTS**

The reaction of pyruvate with H$_2$O$_2$ at neutral pH values was confirmed by measuring CO$_2$ output manometrically in a Warburg apparatus using the necessary controls to recover the CO$_2$ retained in the broth. CO$_2$ was also evolved when broth irradiated by the ultraviolet lamp was immediately mixed with pyruvate; controls with unirradiated broth were negative. Pyruvate was shown to protect bacterial cells from the lethal action of hydrogen peroxide. When a buffered suspension of *M. aureus* was exposed to 20 ppm hydrogen peroxide in the presence and absence of 0.5 per cent pyruvate and aliquots were removed at measured time intervals to tubes containing sterile catalase for inactivating residual peroxide, a graph of the plate counts of the survivors resulted in the curves presented in figure 1. In further experiments pyruvate afforded similar protection to both *E. coli* and *M. pyogenes* against the lethal and the mutagenic action of irradiated broth if the pyruvate was present in the broth during ir-
radiation. However, if the pyruvate was added some time after irradiation, no protection resulted confirming our previous observations (Wyss et al., 1948) that the mutagenic agent was not hydrogen peroxide itself but rather some less reactive compound, possibly an organic peroxide formed in the broth (table 1).

Figure 2. Protection by pyruvate of buffered suspensions of E. coli against exposure to ultraviolet. Survivor curves: A. 0.5 per cent pyruvate present during irradiation. B. Cells soaked in pyruvate and then washed twice before irradiation. C. Control. Mutations curves 1, 2, and 3 correspond with A, B, and C, respectively.

TABLE 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cells (Millions)</th>
<th>Mutants per Million Cells</th>
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</thead>
<tbody>
<tr>
<td>Control broth</td>
<td>773.0</td>
<td>4.5</td>
</tr>
<tr>
<td>Irradiated broth</td>
<td>151.5</td>
<td>43.0</td>
</tr>
<tr>
<td>Pyruvate added after irradiating broth</td>
<td>335.0</td>
<td>21.8</td>
</tr>
<tr>
<td>Broth irradiated with pyruvate</td>
<td>402.5</td>
<td>5.2</td>
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</table>

However, if a buffered solution of pyruvate was irradiated for long periods with the ultraviolet lamp, the pyruvate disappeared as indicated by paper chromatograms made from the solution, and the ultraviolet absorption declined. Yet this irradiated pyruvate solution induced mutations in M. aureus inoculated therein although the effect was not as large as occurred in similarly irradiated nutrient broth (table 2).
The effectiveness of pyruvate in protecting *E. coli* from the lethal action of direct exposure to ultraviolet light is shown in figure 2. The washed cells were reduced sharply in the absence of pyruvate, but in the presence of pyruvate a much greater resistance is demonstrated. Soaking the cells in 0.5 per cent pyruvate and then washing twice with buffer to remove the pyruvate before irradiating in buffer, resulted in only a slight increase in the resistance of the cells to ultraviolet irradiation. A study of the streptomycin-resistant mutants in the survivors showed the usual increase with irradiation dose in the absence of pyruvate, but in the presence of pyruvate, equivalent exposure to ultraviolet gave no significant induction of mutants. However, preliminary treatment of bacteria with pyruvate followed by careful washing, consistently yielded cell suspensions which were more readily mutated by ultraviolet irradiation. This may be related to the mutagenic action of irradiated pyruvate as shown in table 2.

### TABLE 2

*Effect of irradiation on incidence of Micrococcus aureus mutants in culture subsequently suspended therein*

<table>
<thead>
<tr>
<th>TREATMENT OF MEDIUM</th>
<th>MUTANTS PER MILLION CELLS</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>n/200 PO&lt;sub&gt;4&lt;/sub&gt; buffer</td>
</tr>
<tr>
<td>Unirradiated</td>
<td>1.2</td>
</tr>
<tr>
<td>Irradiated, 45 minutes</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Pyruvate solutions show some absorption of ultraviolet that may account for part of the result, but the protection was greater than we obtained with nutrient broth which had a higher absorption throughout the 2,200 to 3,200 A range. Since it is difficult to determine which portion of the effect should be ascribed to absorption, the experiments were repeated with x-radiation in which absorption by pyruvate does not occur. From the apparatus employed the organisms received radiations at 8 Roentgens per second or 28,800 Roentgens per hour. The protection rendered by pyruvate is shown in figure 3. The induction of streptomycin-resistant mutants increased with irradiation dose but in the presence of pyruvate little increase in mutation resulted. This experiment was confirmed both with *M. aureus* and with *E. coli* and with several samples of purified pyruvate.

The addition of pyruvate to bacterial cells after irradiation injury did not give recovery or interfere with the induced mutations. Modification of the recovery medium can modify materially the plate counts of heat treated bacterial suspensions. Roberts and Aldous (1949) have reported some analogous effects with ultraviolet treated cells, but with *E. coli* one commonly obtains greater survival of cells injured by ultraviolet if the plating is carried out on a simple, chemically defined medium rather than on peptone agar. This could be due to the sensitivity of injured organisms to the toxic factors in peptone described
by Foster et al., (1950). We have trained a strain of B. anthracis, an organism with relatively complex nutritional requirements to grow in Gladstone's (1939) medium from which we have omitted a number of amino acids. Yet when the culture is irradiated, a very low count is obtained unless these amino acids are restored to the plating medium. The organisms thus recovered are not biochemically deficient mutants. Pyruvate in the recovery medium does not modify the plate counts of cell suspensions partially killed by ultraviolet or x-radiation.

The addition of pyruvate to cells undergoing irradiation may protect them by any of several mechanisms: (1) it may eliminate peroxide formed during the irradiation process, (2) it may lower the oxygen tension in the suspension by providing a readily usable substrate for oxidative enzymes, (3) it may protect necessary sulphhydryl groups on enzyme and gene proteins by modifying the state of reduction inside the cell.

SUMMARY

The presence of pyruvate in a bacterial suspension protects the cells against the lethal and the mutagenic action of ultraviolet and x-rays and against the lethal action of hydrogen peroxide. Upon prolonged exposure to ultraviolet, pyruvate solutions become toxic and mutagenic. The addition of the pyruvate after exposure of the cells or its presence in the plating medium does not modify the biological response to the radiations. In organisms lacking in synthetic ability a rich recovery medium may give higher counts of cells injured by ultraviolet.

REFERENCES


