# Technical Papers

## Oxygen Protection against Ionizing Radiations[1]

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No clear-cut case of protection of organisms against ionizing radiations by oxygen has ever been reported. Numerous reports have indicated a sensitization to ionizing radiations in the presence of oxygen, with a corresponding protection against these radiations in an oxygen-free environment. Some reports have indicated that oxygen is without effect. The literature is summarized by Patt (*1*), with explanations of the most probable mechanisms involved. Alper (*2*) has reported that during x-irradiation of oxygen-free suspensions of phage S13, inactivation proceeded initially at a rate

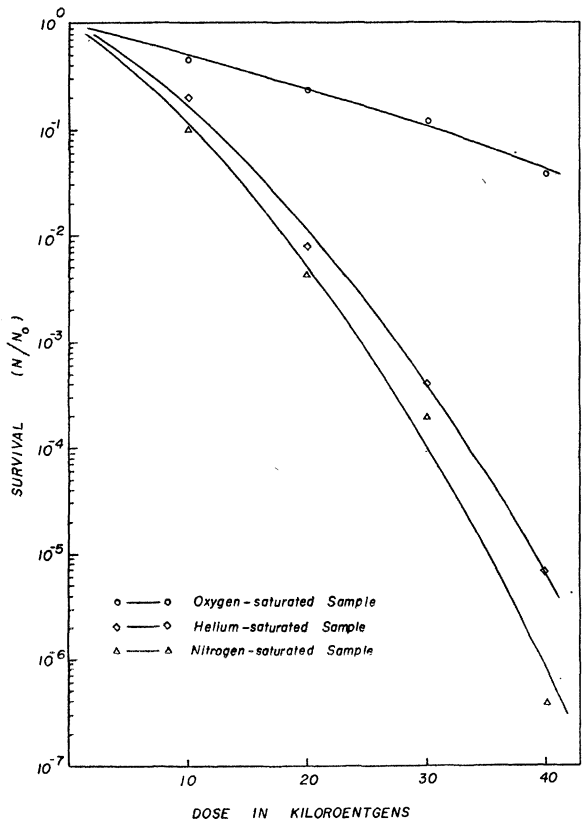


FIG. 1. Survival of bacteriophage T1 plotted as a function of dose of x-rays. The viruses were suspended in saturated solutions of oxygen, helium, and nitrogen.

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[2] We express gratitude to Professor Milton Burton of the Radiation Chemistry Project at Notre Dame for use of the cobalt-60 gamma source.

TABLE 1. Survival ($N/N_0$) of bacteriophage T1 in inorganic salt solutions after exposure to cobalt-60 gamma radiation. The viruses were irradiated in sealed glass vials for 8 min. The dose rate was 4300 r/min, giving a total dose of 34,400 r, as determined by the ferrous ammonium sulfate method.

| Solution | | Oxygen-rich | Oxygen-free |
|---|---|---|---|
| $(NH_4)_2SO_4$ | 1 *M* | $3.1 \times 10^{-1}$ | $2.3 \times 10^{-1}$ |
| | $10^{-2}$ *M* | $4.1 \times 10^{-1}$ | $3.2 \times 10^{-3}$ |
| $NaNO_2$ | 1 *M* | $6.1 \times 10^{-1}$ | $4.0 \times 10^{-1}$ |
| | $10^{-2}$ *M* | $5.8 \times 10^{-1}$ | $5.3 \times 10^{-1}$ |
| | $5 \times 10^{-4}$ *M* | $6.0 \times 10^{-1}$ | $5.2 \times 10^{-1}$ |
| $NH_4NO_3$ | 1 *M* | $4.3 \times 10^{-1}$ | $7.7 \times 10^{-2}$ |

which was certainly no slower than that observed in aerated suspensions, whereas the inactivation rate became greater in aerated suspensions when reaction with $H_2O_2$ became perceptible.

In the present research, bacteriophage T1, specific for *Escherichia coli,* strain B, was irradiated in an aqueous medium made rich with oxygen by bubbling pure oxygen through the suspending medium, or in a medium depleted of oxygen by bubbling purified nitrogen through the medium. The stocks of T1 had been grown in a chemically defined medium utilizing lactic acid as the only organic source of carbon. The cell-free lysates were adjusted so that they contained $3 \times 10^{10}$ particles/ml. The suspension prepared for irradiation consisted of a $5 \times 10^{-3}$ concentration of this lysate in doubly distilled water. All surviving fractions of irradiated phage, $N/N_0$, represent the ratio of the number of irradiated survivors to the number of phage particles treated in all respects exactly the same, except for irradiation. Dilution and plating procedures followed the methods outlined by Adams (*3*).

Typical results for 100-kvp x-rays, filtered by 5 mm equivalent of aluminum and 1 mm of Pyrex glass, are depicted in Fig. 1. The dose rate was adjusted to 1000 r/min, the dose was determined by the oxidation of ferrous ammonium sulfate (*4*); 40 min constituted the longest irradiation period. To check the possibility of harmful or protective components in either the nitrogen or the oxygen, all experiments were run in triplicate with two different sources of nitrogen and oxygen, and, as a further precaution, helium was substituted for nitrogen in a series of three experiments. The results with helium show effects of the same order of magnitude as with nitrogen. Although slightly higher, the results are not considered significantly different. The order of magnitude of the results appears to preclude the possibility that at higher doses the rate of inactivation in the presence of oxygen might proceed at a rate even approaching that in the absence of oxygen.

The phenomenon has been further verified by irradiation with cobalt-60 gamma rays of phage suspensions in distilled water and in a large number of solutions

of inorganic salts. In the tests utilizing inorganic salts, which were added singly to the phage-water suspensions in various concentrations, it has been possible to obscure the oxygen effect or to bring it out sharply, as indicated in the few examples given in Table 1. A discussion of the implications involved with the salts will be presented in detail later.

The results of these experiments raise questions of fundamental importance in radiation biology: Is this effect specific for bacteriophage T1, or is the basic phenomenon reported here obscured in organisms of more complex organization? Does the presence or absence of oxygen alter the phage particles in some way, or is the effect to be explained in terms of suppression or enhancement of certain products of irradiated water? These and related problems are under consideration.

### References


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## Adrenocorticotropic Activity of Nonmammalian Origin


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In the course of establishing the specificity of their now standard bioassay for the adrenocorticotropic hormone (ACTH), Sayers, Sayers, and Woodbury (*1*) administered extracts of liver, brain, and spleen of hypophysectomized rats without affecting the adrenal ascorbic acid concentration of the assay animals. Subsequently, Richards and Sayers (*2*) found that the kidney of intact rats contains a substance which depletes the adrenal ascorbic acid content of hypophysectomized rats. They also found that the kidney accumulates hormonal activity following intravenous administration of rat ACTH. Jailer and Knowlton (*3*) and Opsahl and Long (*4*) have presented evidence, including measurements of adrenal ascorbic acid depletions, for the presence of a type of ACTH activity in human placenta. The presence of adrenal ascorbic acid depleting activity in human placenta was confirmed by one of us (JWN). Recently, it has been observed in this laboratory that ACTH-like activity is present in extrapituitary, nonmammalian sites. About 140 source materials, mostly of microbial origin, have been examined,[1] and we wish to report one of the most interesting developments at this time.

This study was initiated as a result of the finding that the administration to hypophysectomized rats of a 1 mg/100 g body weight dose of an antibiotic feed supplement derived from bacitracin fermentation residues[2] caused a significant depletion of adrenal ascorbic acid concentration. On successive days, average depletions of 84 and 80 mg of ascorbic acid/100 g adrenal tissue were obtained; five rats were employed in each assay.[3]

That there was a chemical resemblance between the ACTH-like activity found in the feed supplement and the pituitary hormone was indicated by the fact that the activity could be extracted and concentrated by the acid-acetone method of Lyons (*5*). This is probably the most commonly employed method for extraction and initial purification of pituitary ACTH. The acid-acetone powder so obtained could then be fractionated with ammonium sulfate to achieve a tenfold concentration of activity.

The ACTH-like activity of the feed supplement was not due to bacitracin polypeptides, judging by the fact that fractions representing stages of increasing bacitracin purity were less potent in adrenal ascorbic acid depletion. When it was noted that an approximately quantitative extraction of the activity from the source material could only be achieved when the solids were first autoclaved with dilute sulfuric acid (a procedure that does not inactivate mammalian ACTH) prior to the acetone treatment, it seemed probable that the ACTH-like activity might be associated with the bacterial organism of the fermentation. Consequently, it was decided to examine some isolated bacterial cells for ACTH-like activity.

*Procedure.* The organism to be studied was transferred from a stock culture to 100 ml of beef-extract-peptone broth and placed in an incubator at 37.5° C for 1 day. An aliquot (1 or 2 ml) of suspended growth was transferred to 200 ml of fresh broth, and the incubation was continued 4 to 5 days. The cellular material was then collected by centrifugation and suspended in dilute sulfuric acid (0.01 or 0.1 normal), employing 3 to 10 vol of acid per volume of packed cells. The resulting suspension was autoclaved at 15 lb pressure for 20 min. After cooling, the suspension was centrifuged, and the supernatant solution was administered in the Sayers bioassay at 0.5 ml/100 g body weight.

In this bioassay, one adrenal is first removed from the test animal (a hypophysectomized rat) prior to the administration of the sample to be tested. The test dose is then administered and, after a suitable time interval, the paired adrenal is removed. Ascorbic acid determinations are then performed on each adrenal, and the depletion resulting from the substance administered is the difference in concentration of ascorbic acid between the animal's own adrenals.

*Results.* The data obtained from the bioassays are presented in Table 1.

In interpreting these data, the following considerations should be kept in mind: (a) In our experience, administration of acidified saline solution, as employed

[1] Dr. G. M. Savage of our Antibiotics Research Department supplied many of the antibiotic beers which were studied.

[2] We are indebted to Dr. W. G. Bywater, of the S. B. Penick Co., who provided the spray-dried residues obtained from filtration of the fermentation broth.

[3] We wish to thank S. C. Lyster and his group for the bioassays. John Karnemaat assisted in the bacteriological work.