the line produced by digested RHP. The remainder of this paper is devoted to description of the reactions of hyaluronidase-digested RHP with antibody to RHP or antibody to human serum; it should be noted that similar results were obtained with hyaluronidase-digested PHP.

When hyaluronidase-digested RHP was studied with a potent antibody to human serum, a single precipitin line was obtained; this made it particularly important to show that the antigenic component was firmly bound in RHP and was not simply a serum protein retained as a contaminant. Evidence for the firm combination of hyaluronate and all the protein in RHP has been reported in detail (*4*); the major line of evidence is the identical electrophoretic mobility of hyaluronate and protein over a wide *p*H range (5.4 to 10.6). After zone electrophoresis at *p*H 7.4, RHP was isolated, digested with hyaluronidase, and studied by immunoelectrophoresis with antibody to RHP and antibody to human serum. A single precipitin line still appeared, indicating that the antigenic component was firmly combined to the hyaluronate. In an attempt to reproduce the conditions in pathological synovial fluids, 1 ml of NHP (3 mg/g) and 2 ml of serum from a patient with active rheumatoid arthritis were mixed and incubated at 37°C for 90 minutes. The NHP, after reisolation and subsequent digestion with hyaluronidase, showed no precipitin lines with antibody to RHP or antibody to human serum (Fig. 1). These results constitute strong evidence that the antigenic properties of RHP are not caused by a serum protein contaminant.

Since the antigenic component in RHP appeared to be related to a serum protein, the reaction of human serums with antibody to RHP was studied by immunoelectrophoresis. Normal as well as rheumatoid serums formed a single line identified on immunoelectrophoresis as an α-globulin (Fig. 2). On agar double diffusion this line formed a reaction of complete fusion with hyaluronidase-digested RHP. Antibody to RHP absorbed with human serum no longer formed a precipitin line with digested RHP.

Thus RHP contains a protein immunologically closely related to, if not identical with, an α-globulin in serum. Tests with specific antiserums to several α-globulins show that this protein is not ceruloplasmin, haptoglobin, or α_2-macroglobulin. The α-globulin is present in normal synovial fluid but passes through the 0.1μ Millipore filter. Thus the α-globulin is not combined with hyaluronate in normal synovial fluid, and it is not possible to combine NHP and the α-globulin in vitro by adding rheumatoid serum to NHP. In the synovial membrane of pathological joints the condition of the hyaluronate may be altered so that it combines with the α-globulin. The antigenicity of PHP described in this paper is due to the presence of this α-globulin which accounts, at least in part, for the higher protein content of PHP (*4*). Although the unusual physical properties of PHP are dependent upon its higher protein content (*4*), the exact role of this α-globulin in determining these interesting physical properties is not yet clear (*5*).

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References and Notes

1. A. G. Ogston and J. E. Stanier, *Biochem. J.* **52**, 149 (1952).
2. C. C. Curtain, *ibid.* **61**, 688 (1955).
3. J. Sandson and D. Hamerman, *J. Clin. Invest.* **41**, 1817 (1962).
4. D. Hamerman and J. Sandson, *ibid.* **42**, 1882 (1963).
5. Supported by USPHS research grant AM-04804.
* Investigator, Health Research Council (contract I-157), City of New York.

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Radiation Action on DNA in Bacteria: Effect of Oxygen

Abstract. *In* Escherichia coli *cells ionizing radiation produces a degradation of DNA to approximately 50 percent of the total amount originally insoluble in trichloroacetic acid. There is also a reduction of synthesis. Oxygen inhibits the degradation process by a dose reduction factor of 4 and the synthesis process by a factor of approximately 1.5. Thus, at relatively low doses, radiation action on bacteria is very probably mediated through the DNA.*

One of the best-established modifiers of the action of ionizing radiation on living cells is dissolved oxygen. Gray *et al.* (*1*), Howard-Flanders (*2*), and others have reviewed this subject. The effect of oxygen on the uptake of phosphorus and sulfur in cells of *Escherichia coli* was observed by Pollard and Macaulay (*3*). Among the effects sensitive to ionizing radiation is the degradation of DNA. This was first shown in microorganisms by Stuy (*4*), and work on the degradation of DNA in *E. coli* has been reported by Miletic, Kucan, and Novak (*5, 6*). The fact that DNA synthesis continues after radiation at a reduced rate was shown by Pollard and Vogler (*7*) and independently by Billen (*8*).

Since any effect at all of radiation on DNA in the cell would be expected to be damaging, the question arises whether the "oxygen effect" is also apparent in degradation and in reduced synthesis of DNA. A strong oxygen effect has been found for degradation and a lesser effect for the reduction of synthesis.

The degradation of DNA can be very readily observed with a thymine-requiring mutant of *E. coli.* Such a thymine-requiring mutant can be given labeled thymine, and the subsequent fate of the label can be studied by determining whether the thymine is incorporated into large hydrogen-bonded material, which is precipitated by trichloroacetic acid (TCA), or whether it is released into the soluble fraction. By a combination of labeling of *E. coli* 15 T-L-, a mutant requiring both thymine and leucine for growth (*9*), with thymine, and of subjecting cells to radiation, the degradation of the DNA has been estimated directly by observing that part of the label which appears in the TCA insoluble fraction of the cell extract.

Also if the cells are treated with oxygen by bubbling the gas through the suspension and are then irradiated with Co^{60} γ-rays, the effects of radiation are increased fourfold as compared with cells that had been similarly exposed to nitrogen. The dose-reduction factor is defined as the ratio of doses that produce the same observed effect, the factor being 4 in this case. In Fig. 1 we show the results of two experiments in which 10- and 20-kr doses were given. Here the cells were first grown on medium supplemented with C^{14}-thymine through several periods of division; just prior to the experiment the cells were "chased" with "cold" thymine for 15 minutes. In such cells the pool of thymine as measured in our laboratory corresponds to 10 minutes' growth time. Thus the 15-minute "chase" essentially eliminates the whole pool of thymine and leaves only labeled thymine in the DNA. The time

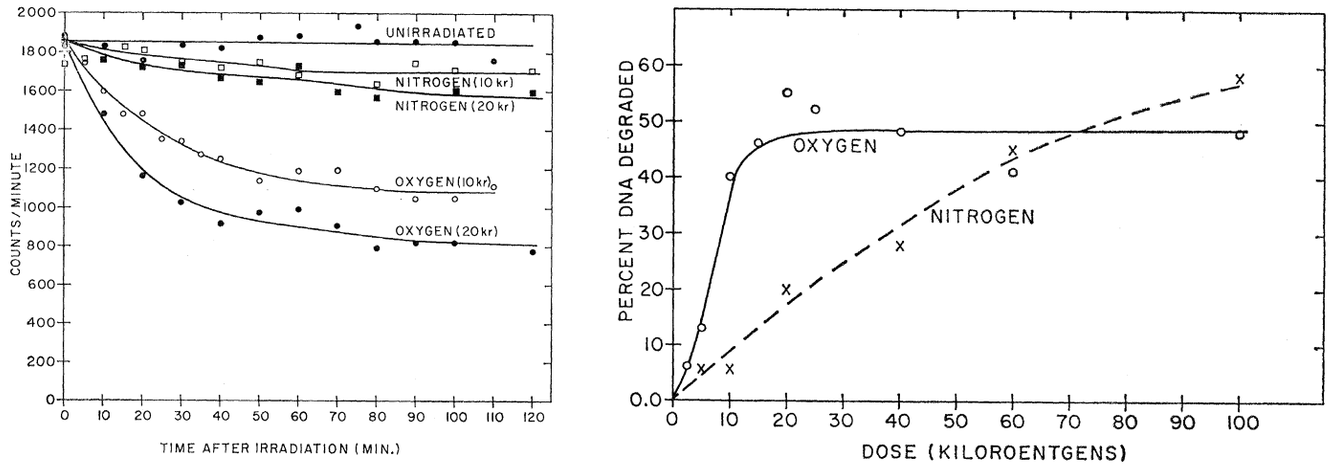

Fig. 1 (left). Time course of degradation of *Escherichia coli* DNA irradiated in the presence of oxygen and nitrogen with 10 kr and 20 kr of Co^{60} γ-rays. Cells were labeled with C^{14}-thymine until 15 minutes before irradiation, when it was replaced by normal thymine until and after irradiation. The amount of C^{14} in the fraction insoluble in cold 5 percent TCA is plotted as a function of time for unirradiated cells, for cells irradiated after exposure to nitrogen bubbling, and for those irradiated after oxygen bubbling. Fig. 2 (right). Amount of DNA degradation in the presence of oxygen and nitrogen for various doses up to 100 kr. Both curves reach a value near 50 percent degradation. As seen from the initial slopes, there is a dose reduction factor of 4 in the presence of oxygen.

course of the process is observed by removing samples of 1 ml at intervals. The samples are then treated with 1 ml of cold 10 percent TCA for 1 hour, and the precipitate so formed is filtered on a Schleicher and Schuell type B-6 filter and washed with 10 ml of Roberts' C minimal medium (*10*). Cells which have not been irradiated continue to have a constant fraction of their labeled thymine in the insoluble fraction (Fig. 1). Cells which have been irradiated lose the C^{14}-label from the fraction insoluble in TCA. This loss reaches a fixed amount for each dose. In the presence of nitrogen the effect is very much reduced as compared to the effect in oxygen. In control experiments the label appeared in the fraction soluble in TCA.

In experiments (similar to those described in Fig. 1) on the effect of radiation dosage a maximum of approximately 50 percent of the DNA can be degraded. The relative effect of treatment with oxygen and nitrogen is shown in Fig. 2, where the fraction degraded is plotted as a function of dose. The initial slopes of the lines differ by a factor of 4 in favor of oxygen.

When labeled cells were heated to 70°C for 10 minutes, a time which should inactivate deoxyribonuclease, subsequent irradiation and incubation resulted in no degradation at all. We conclude that the action of radiation in causing degradation is partly due to a sensitization to some kind of enzyme action.

Synthesis of DNA can be studied by continuing to add labeled thymine during the process of growth after radiation. Figure 3 shows that with low doses of radiation an appreciable synthesis continues. The dashed lines represent the original data, obtained by the method described, and show a

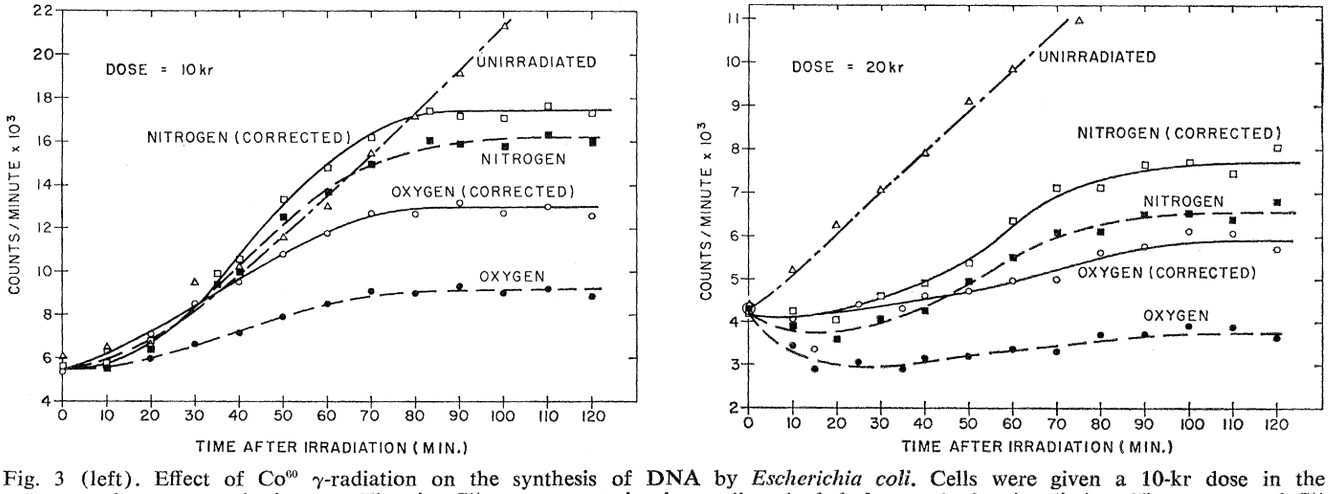

Fig. 3 (left). Effect of Co^{60} γ-radiation on the synthesis of DNA by *Escherichia coli*. Cells were given a 10-kr dose in the presence of oxygen and nitrogen. Thymine-C^{14} was present in the medium both before and after irradiation. The amount of C^{14} in the fraction insoluble in cold TCA increases after irradiation until a time approximating the division time, after which there is no further increase. Correction can be made for the degradation of DNA by using the information of Fig. 1. The lines so corrected are shown as continuous lines. Fig. 4 (right). The effect of 20 kr of Co^{60} γ-radiation on the synthesis of DNA by *E. coli*. Conditions and corrections are the same as in Fig. 3.

steady increase in synthesis when the irradiation is performed in nitrogen-bubbled medium, followed by a break to a lower rate of synthesis at about 60 minutes. The rate was apparently much less when the irradiation took place in oxygen. The results show that this type of experiment includes both the degradation after radiation and also synthesis. If correction is made for the amount degraded (data of Fig. 1), then the corrected (solid) lines are obtained. Synthesis continues at an unchanged rate for both conditions of irradiation, but the cessation of synthesis occurs earlier when the medium is oxygenated. In Fig. 4 we show the same kind of data for a dose of 20 kr. The effect is much more marked; there is a depression of synthesis early in the experiment and a subsequent leveling off. A comparison of Figs. 3 and 4 indicates that the effect produced, in oxygen, by 10 kr would have been approximately duplicated by 15 kr in nitrogen. Thus the dose reduction factor is of the order of 1.5 and certainly very much less than that found for the process of degradation. It is interesting that the cessation of synthesis seems to be associated with the division time of the culture, occurring a little later.

When the cells were irradiated on filters, a condition corresponding to a full oxygenation, the label was given only after irradiation so that correction for degradation was not necessary; the results were in agreement with the aforementioned corrected values for oxygen.

Since these effects are observed at doses which are of the same order as those observed for the loss of colony-forming ability, the most commonly observed effect of ionizing radiation—the dose for 37 percent survival for these cells is 3 kr in oxygen and 12 kr in nitrogen—it is quite attractive to suppose that one of the primary damaging actions of ionizing radiation might be on the DNA. The oxygen effect is characteristic of the effect of ionizing radiation on living cells, and, because it is observed so clearly in the case of DNA degradation, the hypothesis that a considerable part of the oxygen effect is due to this degradation is attractive. The lesser effect of oxygen on the synthesis of DNA indicates the presence of a second process. The fact that the amount of DNA degraded when radiation occurs with oxygen present does not exceed 50 percent suggests that a part of the DNA is especially sensitive to this type of radiation. We suggest that one strand of the DNA is more sensitive than the other.

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References and Notes

1. L. H. Gray, A. D. Conger, M. Ebert, S. Hornsey, O. C. A. Scott, *Brit. J. Radiol.* **15**, 72 (1959).
2. P. Howard-Flanders, *Advan. Biol. Med. Phys.* **6**, 553 (1958).
3. E. Pollard and P. Macaulay, *Radiation Res.* **15**, 120 (1961).
4. J. H. Stuy, *ibid.* **14**, 57 (1961).
5. B. Miletic, Z. Kucan, L. J. Sasel, *Intern. J. Rad. Biol.* **7**, 141 (1964).
6. B. Miletic, Z. Kucan, D. J. Novak, *Nature* **202**, 106 (1964).
7. E. Pollard and C. Vogler, *Radiation Res.* **15**, 109 (1961).
8. D. Billen, *Biochim. Biophys. Acta* **72**, 608 (1963).
9. The *E. coli* 15 T-L- was obtained from Frederick Forro of Yale University.
10. R. B. Roberts, D. B. Cowie, P. H. Abelson, E. T. Bolton, R. J. Britten, "Studies of biosynthesis in *E. coli*," *Carnegie Inst. Washington, Publ. No. 607* (1953).
12. We thank Dr. Stan Person for very helpful discussions. Work supported by AEC contract AT(30-1)2804.

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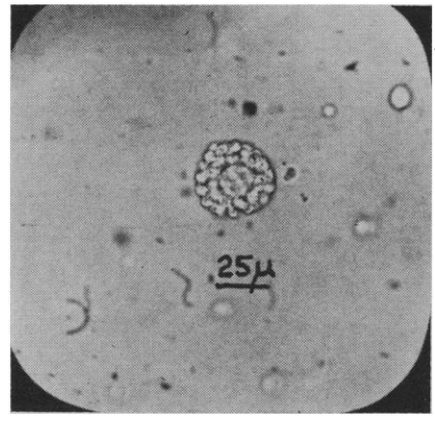
Enzymatic Dissection of the Mammalian Ovary

Abstract. *Incubation of the ovaries of rats and mice with the proteolytic enzyme "pronase" caused the step by step dispersal of cellular elements from the tissue. The first subunits to be liberated were the outermost follicles or corpora lutea or both. Next, these subunits were dispersed. It is possible to obtain isolated single follicles in all stages of development: corpora lutea, isolated cell layers from within follicles, suspensions containing free cells, and ova in all phases of maturation.*

Proteolytic enzymes have been utilized for the dispersal of both embryonic and adult tissues (*1*). Pronase, a partially purified protease from the actinomycete *Streptomyces griseus* (*2*), has been used for removal of the zona pellucida (*3*), and in the dispersal of several types of tissue (*4*).

To obtain isolated follicle cells, the ovaries of 30 rats and mice were



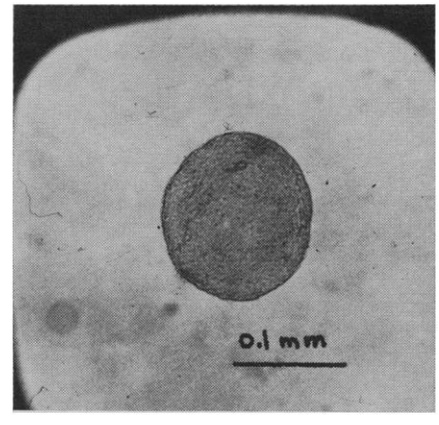


Fig. 1 (left above). Isolated primary follicle, unstained. The ovum is surrounded by follicle cells and can be seen in the center of the structure.

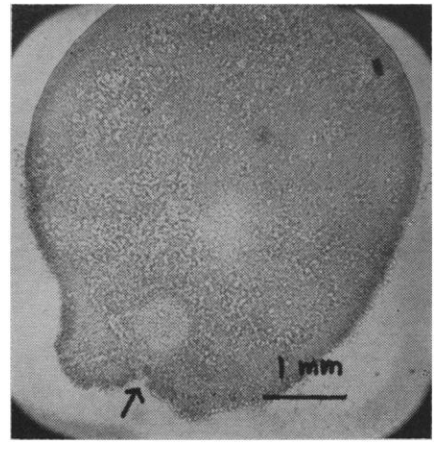


Fig. 2 (right above). Growing follicle, unstained. The size corresponds to that of a follicle at the beginning of antrum formation. The ovum is not visible, owing to the number of cell layers making up the structure.

Fig. 3 (left). Graafian Follicle, unstained. The follicle is in the process of dispersal (arrow). The ovum can be seen as the pale area near the indicated zone of dispersal.