

Studies on the Recovery of X-Irradiated Bacteria

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X- und UV-irradiation at lower dose-values result in the impairment of bacterial division mechanism and this can be restored to the original working condition under the influence of bacterial extract either on agar plates¹ or both on solid as well as in liquid media². The restoration phenomenon was found to be dependent on soluble protein component of the extract², but independent of various plating media³. In this communication I have studied the influence of protein synthesis of the test as well as donor organisms on the observed extract-promoted recovery. I have also studied whether X- and UV-exposures of the bacterial extract affected the recovery process as did heat exposures².

For the present experiments *E. coli* B cells were used both as test as well as donor organisms. The details of bacterial growth, preparation of, and working with bacterial extract were given in a previous publication². Protein synthesis of bacteria was arrested by using chloramphenicol (CM) as the inhibitor of protein at a concentration of 100 μg per ml. X-ray was obtained from a Muller MG 150 tube run at 80 kV, 9 mA. The X-ray dose as measured by the method of FeSO_4 -dosimetry was 6 krad per min. Ultraviolet light was supplied from a G.E. germicidal lamp, the dose being 7.5 ergs/ mm^2 sec. UV-dose was determined by UV-actinometry⁴. Viable bacterial number was determined by plating technique on nutrient agar plates. Visible colonies were counted for viable number after incubating the agar plates for 18 hrs at 37°C.

Log-phase *E. coli* B cells were allowed to grow for half an hour in tris-glucose media (TG) containing CM at 100 $\mu\text{g}/\text{ml}$. They were then X-irradiated and incubated for 1 hr in optimal concentration of extract of *E. coli* B. These bacteria failed to show any rise in X-ray survivors. The results are shown in Fig. 1. Again, in another aspect of the study, log-phase cells grown in TG media were exposed to X-rays and subsequently held at 37°C in an extract prepared from cells whose protein synthesis was inhibited for half an hour prior to their lysis. It may be observed that recovery of X-irradiated cells was also found to be absent under the circumstances (see Fig. 1).

Fig. 2 shows how X-irradiation of the extract influenced the increase in survival of X-irradiated bacte-

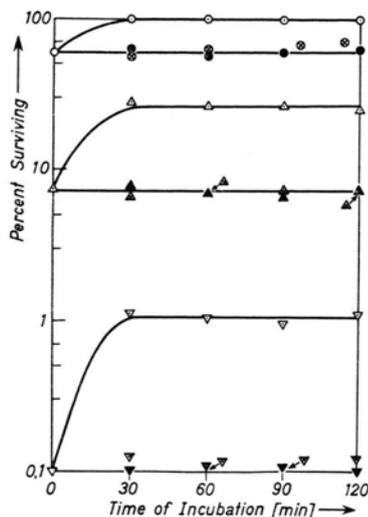


Fig. 1. Influence of protein synthesis of both test and donor organisms on the extract-promoted recovery of X-irradiated bacteria. Protein synthesis not inhibited prior to irradiation with \odot : 1 kr, \triangle : 6 kr, ∇ : 12 kr of X-rays. Protein synthesis of the test (\bullet , \blacktriangle , \blacktriangledown) and the donor (\otimes , \triangleleft , \triangleright) bacteria inhibited prior to irradiation of the test bacteria with X-ray doses, \bullet , \otimes , 1 kr, \blacktriangle , \triangleleft , 6 kr, \blacktriangledown , \triangleright , 12 kr.

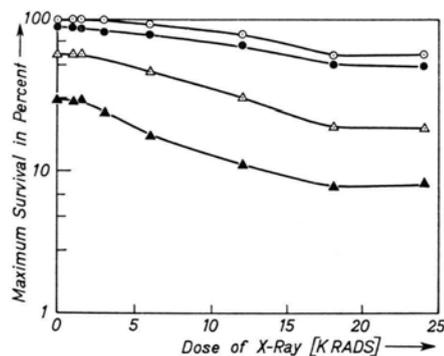


Fig. 2. Influence of X-irradiation of bacterial extract on its ability to increase the survivors at X-ray doses \odot : 1 kr, \bullet : 1.5 kr, \triangle : 3 kr, \blacktriangle : 6 kr.

ria, which was normally observed by unexposed extract². X-ray doses used for the test bacteria and extract ranged from 1 to 6 krad and 1 to 48 krad respectively. Use of dose-range 1–6 krad for test bacteria was chosen on the ground that the increase in survival of X-irradiated bacteria due to incubation in extract was much more prominent for irradiation with doses up to 6 krad. It is seen from the results that extract exposed to X-ray doses less than 6 krad gave normal recovery in X-irradiated bacteria, when

¹ W. D. FISHER, H. I. ADLER, F. W. SHULL, JR., and A. COHEN, *J. Bacteriol.* **97**, 500 [1969].

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⁴ C. G. HATCHARD and C. A. PARKER, *Proc. Roy. Soc. [London]*, Ser. A, **235**, 518 [1956].

incubated in its presence i.e., the exposed extract gave results as if it was not irradiated with X-rays. But when the extract was irradiated with 6 krads and higher doses of X-rays, the increase in the survivors of X-irradiated population (or recovery) was found to be affected. The time of incubation and concentration of extract² were 1 hr and 1662.5 $\mu\text{g}/\text{ml}$ respectively in all the experiments. The suppression in recovery began when extract was irradiated with 6krads, complete inhibition of the observed extract-promoted recovery occurred at 18 krads.

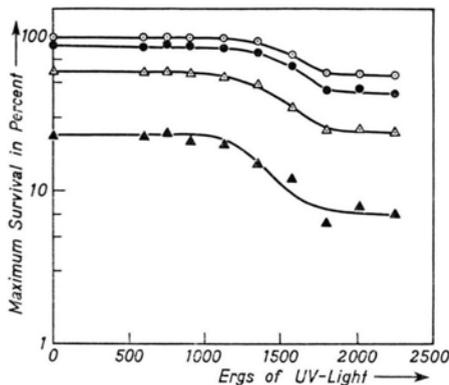


Fig. 3. Influence of UV-irradiated extract on the survival number at X-ray doses \odot : 1 kr, \bullet : 1.5 kr, \triangle : 3 kr, \blacktriangle : 6 kr.

Fig. 3 represents the influence of UV-irradiated extract on its ability to promote recovery in X-irradiated bacteria. Increase in survival was studied in this case

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in otherwise identical conditions. It may be observed from Fig. 3 that some increase in X-ray survivors or recovery was obtainable in the cases where extract was exposed to UV-light below 1350 ergs per mm^2 . Beyond this dose, recovery was gradually inhibited. At about 2000 ergs per mm^2 , it was completely inhibited.

Out of the results presented above emerged an interesting feature of the extract-promoted recovery that protein synthesis of both donor and test bacteria was needed. If the synthesis of protein of either system of bacteria is stopped, the recovery is also inhibited thereby. Need of metabolism of the test bacteria for the occurrence of repair of X-ray damage was also stressed by other investigators^{5,6}. Inhibition of recovery of the test organisms in the absence of the protein synthesis of the donor is possible, because active principle supplied by the donor was of the nature of protein, possibly soluble protein². Repair activity contained in the bacterial extract can be destroyed by X-ray, UV-light similar to the destruction of the active component of the extract by heat exposures². There are evidences to suggest that X-ray induced loss in the activity of the extract might be due to the damage to the soluble proteins^{7,8}. Thus results of X- and UV-irradiation of extract simply gave a further confirmation of our previous observation that soluble protein is responsible for extract-promoted recovery².

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On the Nature of Achromatic Lesions ("Gaps") Induced by X-Rays in Chromosomes of *Vicia faba*

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Achromatic lesions or "gaps" are non-staining regions traversing the diameter of the chromatid. Infor-

mation on what is known about the nature of these lesions induced by X-rays in chromosomes of the broad bean *Vicia faba* is supplied by some reviews¹⁻³ and by a few more recent publications⁴⁻¹⁰. Here only two remarkable features of these lesions will be mentioned: their high frequency (they represent, in *Vicia faba* at least, the most frequent microscopically visible chromosomal change) and the fact that most of the gaps are repaired by the time the second post-irradiation mitosis is reached.

Experiments with UV-⁶ and scanning electron microscopy⁷ which we have carried out revealed that at least

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² H. J. EVANS, in: Radiation-Induced Chromosome Aberrations (Ed. S. WOLFF), p. 8, Columbia University Press, New York 1965.

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⁷ W. SCHEID and H. TRAUT, Mutation Res. **11**, 253 [1971].

⁸ W. SCHEID and H. TRAUT, Mutation Res. **12**, 97 [1971].

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a proportion of the gaps studied exhibit two parallel strands. Probably these structures are typical of X-ray induced gaps, i. e. when the strands are not observed this may be because of technical reasons. The results obtained with the UV-microscope⁶ favour the assumption that the strands contain DNA. However, we cannot exclude the possibility that the visualization with UV-microscopy at 265 ± 9 nm is based on the absorption due to protein and not due to DNA. (It seems plausible to assume that the strands have a protein component.) In order to remove this uncertainty the following experiments were performed.

Seeds of the broad bean *Vicia faba* (var. Prolific Longpod) were cultured as described elsewhere⁴. 10-day-old seedlings were irradiated with 100 R X-rays (150 kV) applied in 1 min. The experiments were carried out in such a way (see ref. 4 for details) that most of the gaps were induced in late interphase and early prophase but analysed in metaphase. The usual squash technique is not appropriate to demonstrate the two strands within the gap. (This seems to be the reason why these structures have not been detected earlier.) Therefore we analysed the gaps in chromosomes that were isolated as described earlier⁶ and then stained with the DNA specific Feulgen reagent: One 10-min wash in distilled water, hydrolysis with 6 N HCl for 6–10 min, three 5-min washes in distilled water, staining for 3 h in Schiff's reagent, three 5-min treatments in sulphite bleach¹¹, three 5-min washes in distilled water, alcohol (95%) for 5 min, absolute alcohol for 15 min, drying in vacuum and mounting in distilled water.

Within several gaps induced in chromosomes prepared in this way, the two strands could be seen in the light microscope. Fig. 1 represents an example. Because the Feulgen reaction is DNA specific this finding proves that the strands contain DNA. Possibly the two strands observed by us are identical with the two strands representing the "chromatid skeleton" obtained when (unirradiated) *Vicia faba* chromosomes are treated with trypsin and then stained with the Feulgen reagent^{12, 13}.

Furthermore we stained isolated *Vicia faba* chromosomes with dyes specific both for DNA and RNA, namely the fluorochromes ethidium bromide (a trypanocidal dye)¹⁴ and berberin sulphate (an alcaloid)¹⁵. The biological and irradiation conditions were the same as those of the Feulgen experiments (see above). In the ethidium bromide series a solution of ethidium bromide (0.005%) in 0.1 M tris buffer (pH 7.5) was used. The staining with berberin sulphate was performed as described in ref. 15. In both series some of the chromosomes were treated with ribonuclease before

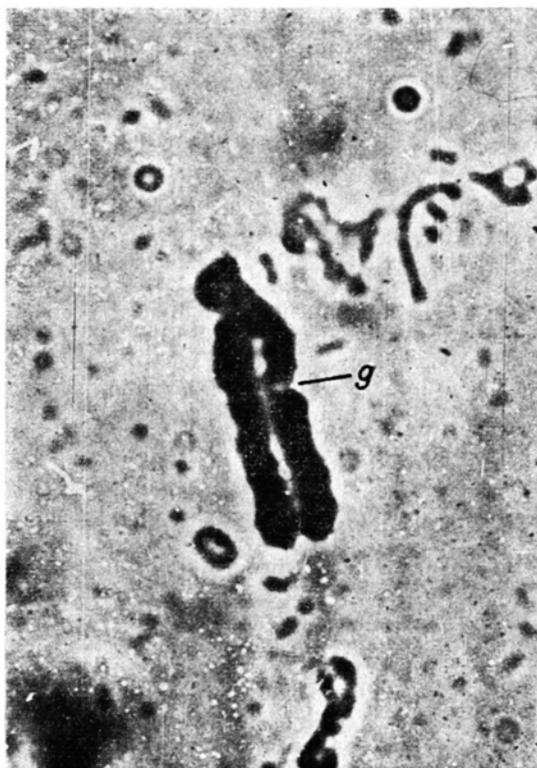


Fig. 1. An X-ray-induced achromatic lesion, "gap" (g), exhibiting two parallel strands as revealed by the Feulgen test. An isolated Feulgen-stained S-chromosome (length: $11.3 \mu\text{m}$) of *Vicia faba* is represented.

staining (see ref. 16). In each kind of experiment, i. e. after staining with ethidium bromide and berberin sulphate, respectively, and that with and without ribonuclease pretreatment, the two strands could be observed under the fluorescence microscope. This confirms our Feulgen results, namely the demonstration that these structures contain DNA. However we were not able to obtain satisfactory microphotographs of the strands visualized by fluorescence.

Altogether we have now demonstrated the existence of the two strands within gaps induced by X-rays in chromosomes of *Vicia faba* in the following ways: by fluorescence microscopy (using ethidium bromide and berberin sulphate), by UV⁶ and scanning electron microscopy⁷, as well as by the Feulgen test.

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