The results show that the $^{32}P_i$ incorporation into the alkaline phosphatase from *Escherichia coli* was highly similar to that into the calf-intestinal enzyme. This supports the conclusion that the $^{32}P_i$ incorporation is related to the mechanism of action of alkaline phosphatases.

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## Failure of ultraviolet-irradiated *Escherichia coli* to produce a cross-reacting protein

Irradiation with ultraviolet light inhibits the ability of cultures of *Escherichia coli* to synthesize active β-galactosidase. This synthesis decreases with increasing radiation dosage at a considerably greater rate than does total protein synthesis (Fig. 1). For example, an irradiation which was sufficient to reduce enzyme synthesis by 80% only reduced protein synthesis by 20%. Alkaline phosphatase, aspartate transcarbamylase, tryptophanase and D-serine deaminase exhibit similar ultraviolet sensitivities[1]. If these enzymes are to be regarded as typical cellular proteins, one wonders why their synthesis should be particularly sensitive to radiation.

One possible explanation is that irradiated bacteria synthesize proteins which are closely related to the enzymes, but are devoid of catalytic activity because of some small structural change. The observation that about half of the radiation-induced β-galactosidase-negative mutants are capable of synthesizing a cross-reacting material which will precipitate antibody to β-galactosidase makes this hypothesis particularly attractive[2]. Alternatively, the irradiated bacteria may simply synthesize a reduced amount of normal enzyme and either no abnormal protein, or some additional protein which, although derived from the enzyme, is devoid of any immunologically detectible structural similarity.

Immunological tests were performed on material produced immediately after irradiation in order to determine if the irradiated organisms now synthesize a cross-reacting material in the place of active enzyme, thus testing the first hypothesis. The test employed detects cross-reacting material by its ability to prevent combination and precipitation of antibody and active enzyme. If enzyme remaining in the supernatant after precipitation is plotted against enzyme input, the point at which residual

enzyme is first detected in the supernatant will be shifted toward the origin in the presence of cross-reacting material. This effect is shown in Fig. 2 in which a curve obtained from an enzyme–antibody precipitation is compared with others in which the enzyme extract was mixed with various proportions of cross-reacting material

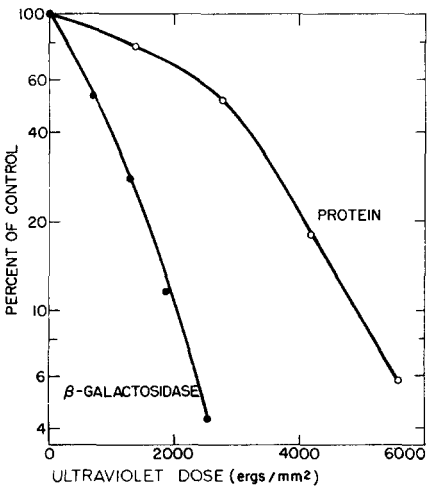


Fig. 1

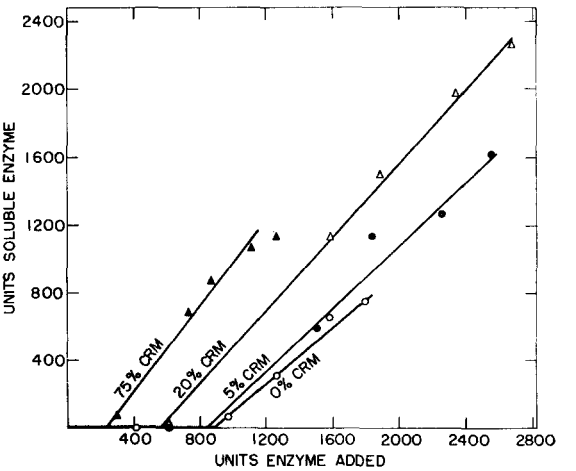


Fig. 2

Fig. 1. Inhibition of β-galactosidase and protein synthesis as a function of ultraviolet dose.

Fig. 2. Immunological demonstration of cross-reacting material (CRM).

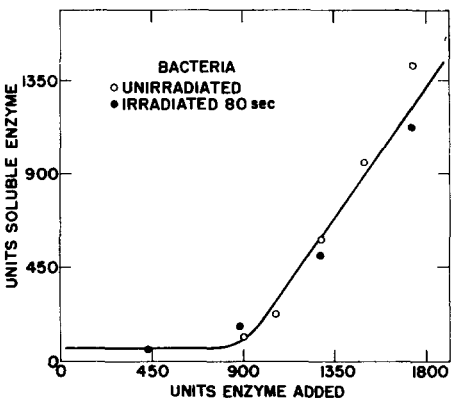


Fig. 3

Fig. 3. Immunological titration of β-galactosidase from unirradiated and irradiated *E. coli*.

extracted from a mutant producing cross-reacting material. This method is sufficiently sensitive to detect cross-reacting material unequivocally in the presence of ten times the antigenically equivalent amount of active enzyme. Under the conditions used, solubilization of the antigen–antibody complex[3] did not occur to any significant extent.

*E. coli* K12, strain 2000a, were grown aerobically with swirling at 37° in a glycerol–salts medium[4] to a concentration of $10^9$ cells/ml. Aliquots (100 ml) of the log-phase culture were irradiated for various times with an ultraviolet sterilizing lamp (15W Sylvania Germicidal-A) with principal output at 2537 Å at an intensity of 35 ergs/mm²/sec. At the highest (approx. 2700 ergs) of several doses used, survival was less than 0.1% and enzyme formation approx. 10% of the level reached in an

unirradiated culture. The bacteria were then diluted with an equal volume of fresh medium and $5 \cdot 10^{-4}$ *M* isopropyl-β-D-galactoside was added to induce β-galactosidase. The cultures were shaken until the absorbancy of the cultures had doubled. They were then centrifuged, frozen, ground with alumina and the enzyme extracted with buffer as described by HU *et al.*[5]. After centrifugation the enzyme-containing supernatants were used in the serological tests.

Rabbit antiserum was prepared by the method described by COHN[6]. β-Galactosidase, extracted from a mutant of *E. coli* (Strain E-16, isolated by Dr. A. NOVICK) which produces the enzyme super-constitutively, was partially purified[5] before use as antigen. The immunological tests were based on a method used by COHN AND PERRIN[7]. Increasing aliquots of bacterial extract were mixed with 0.5 ml of a 1:16 dilution of antiserum in 0.1 *M* phosphate buffer (pH 7) and the volume was brought up to 1 ml with Buffer A (HU *et al.*[5]). Both irradiated and unirradiated bacteria were tested. Controls were run using normal rabbit serum and these were used to determine enzyme input values. After incubation at 37° for 30 min, samples were allowed to stand overnight in the refrigerator to insure complete precipitation. The precipitate was removed by centrifugation and the enzyme remaining in the supernatant was assayed for β-galactosidase activity[8].

Cross-reacting material was not detected in any of the irradiated cultures (Fig. 3). From this result we may conclude that production of an immunologically similar but enzymically inactive protein does not account for a significant part of the disparity between total protein and detectible enzyme synthesized after irradiation (Fig. 1). Apparently any ultraviolet lesion is sufficiently severe to prevent the formation of a slightly damaged but immunologically active protein. Presumably, either a protein differing radically from the original enzyme, *i.e.* immunologically inactive, is formed or the damaged bacterium loses the ability to synthesize any protein related to β-galactosidase. The actual cause of the protein disparity will have to be sought elsewhere.

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