these figures can easily be identified with computer simulated 'diffusion limited aggregates' (DLA)[1,2]. Some nucleation sites have grown to large fractals but there are many more nucleation centres (seen as small dark violet dots in the Figures 1 and 2). The size of fractal has been found to grow with time. Further, it has been noted that larger the amount of Al_2O_3, more are the nucleation sites created. The fractal dimension was calculated from the results shown in Figure 2 following standard procedure[1,2] and found to be ~ 1.69 confirming the DLA mechanism.

Our observations can be explained as follows. In the $(PEO + NH_4I + Al_2O_3)$ films, Al_2O_3 is in the dispersed phase. When Al_2O_3 is added to $(PEO + NH_4I)$ complex, it knocks out the I^- ion from the lattice, which is hanging in the PEO chain, thereby giving I^- centres for nucleation. These knocked out I^- ions make a random walk[8] and if they visit a site next to the centre of nucleation, they are stuck to it and form a two-particle cluster. In a similar manner, more and more particles keep on sticking and one observes the growth of a random object with open branches.

These fractal patterns have been obtained in more than 20 films with different NH_4^+/EO and Al_2O_3 ratios over an extended period of time for studying the dynamics of growth. The results are repeatable. The details would be published later.

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Role of materials released from *Escherichia coli* cells in the catabolite repression of L- arabinose isomerase induced by γ-irradiation

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Inhibition of L-arabinose isomerase synthesizing capacity of *Escherichia coli* by γ-irradiation was withdrawn when the irradiated cells were separated by centrifugation from the suspending medium and were suspended in a fresh growth medium. Further, there was an inhibition of the induced enzyme synthesis in unirradiated cells when suspended in the supernatant from γ-irradiated cell suspension. These findings suggested that some inhibitory material(s) was released from γ-irradiated cells into the suspending medium. Incubation of γ-irradiated cells suspended in fresh growth medium led to an exponential decrease in the enzyme synthesizing capacity, which was not caused by release of any inhibitory material. Cyclic AMP could reverse the inhibition of the enzyme synthesizing capacity in unirradiated cells caused by their suspension in the supernatant from γ-irradiated cell suspension immediately following irradiation suggesting that catabolite repression is the cause of the inhibition. Incubation of γ-irradiated cell suspension released the L-arabinose isomerase-synthesizing system from the catabolite repression.

EARLIER work from this laboratory revealed that γ-irradiation of *Escherichia coli* B/r causes a dose-dependent inhibition of the induced synthesis of L-arabinose isomerase[1]. The inhibition could be partially reversed by cyclic AMP suggesting that catabolite repression caused by lowering of cellular cyclic nucleotide level was partly responsible for this inhibition. The reduction in the cyclic AMP level could be ascribed to a decreased synthesis of the nucleotide[2]. Although cells receiving lower doses (below 180 Gy) recovered from the damage to the enzyme synthesizing system, at higher doses (180 Gy and above) post-irradiation incubation led to further inhibition of the capacity of the cells to synthesize L-arabinose isomerase. This enhanced inhibition was shown to be due to a process other than catabolite repression[1].

Although various authors have shown that nuclear materials are released into the suspending medium following incubation of X-irradiated cells[3,4], no reports are available on the role of materials released from γ-irradiated cells on induced enzyme synthesis. The present study deals with the mechanism of inhibition of L-arabinose isomerase synthesizing capacity of the cells following γ-irradiation with special reference to the involvement of materials released from the irradiated cells in this inhibition.

Escherichia coli B/r (ORNL) was kindly supplied by Dr P. A. Swenson of the Oak Ridge National Laboratory, USA. Cyclic AMP and L-arabinose were

obtained from Sigma Chemical Company, USA. All other chemicals were of analytical grade.

Tris–EDTA treatment of *E. coli* cells was done according to the method described earlier[5]. The method for γ-irradiation has also been described in a previous paper[1].

Induction of L-arabinose isomerase and assay of the enzyme were carried out as described previously[6]. For this, Tris–EDTA–treated cells suspended in minimal medium containing 0.2% glycerol and 0.025% casamino acids at a cell density of 5×10^8 cells/ml were kept at 37° C with constant shaking. L-Arabinose was added as the inducer at a final concentration of 1.33×10^{-2} M. Samples (1 ml) were collected at different time intervals into prechilled tubes containing chloramphenicol at a final concentration of 50 μg/ml to stop further synthesis of the enzyme. To the induced cell suspension (1 ml) one drop of toluene was added. The mixture was vigourously shaken in a vortex mixer and was incubated at 37° C for 5 min. These toluene-treated cells were used for the assay of L-arabinose isomerase.

The incubation mixture (1 ml) for the assay of the enzyme contained 62.5 μmol of Tris. HCl, pH 7.4, 0.5 μmol of $MnCl_2$, 10 μmol of L-arabinose and requisite amount of toluene-treated cell suspension. Incubation was carried out at 37° C for 15 min. The amount of L-ribulose formed was determined by cysteine–carbazole colour test. Absorbancy was measured at 530 nm at 15 min after the addition of carbazole.

One unit of L-arabinose isomerase is defined to be the amount which produces a change in absorbancy of 0.10 cm^{-1} at 530 nm.

Tris-EDTA-treated *E. coli* B/r cells, suspended in minimal medium containing glycerol (0.2%) and casamino acids (0.025%) to have a cell density of 5×10^8 cells/ml, were irradiated with different ^{60}Co γ-ray doses. Immediately after irradiation, cell suspension was centrifuged. The pellet containing cells was resuspended in fresh growth medium The supernatant was used to suspend unirradiated Tris-EDTA-treated cells. The cell density in both the cases was kept at 5×10^8 cells/ml. The capacity of the cells to synthesize L-arabinose isomerase for 60 min in response to the addition of L-arabinose (1.33×10^{-2} M) was determined. The enzyme synthesizing capacity of γ-irradiated cells without separating them from the cell suspension was also determined. The results are presented in Figure 1. As reported earlier[1], L-arabinose isomerase synthesizing capacity of cells decreased exponentially with increasing γ-ray dose with which they were irradiated. When the irradiated cells, after their *separation by centrifugation* from the suspending medium, were resuspended in a fresh growth medium they regained their enzyme synthesizing capacity. The supernatant obtained after centrifugation of γ-irradiated cell suspension inhibited the induced synthesis of L-
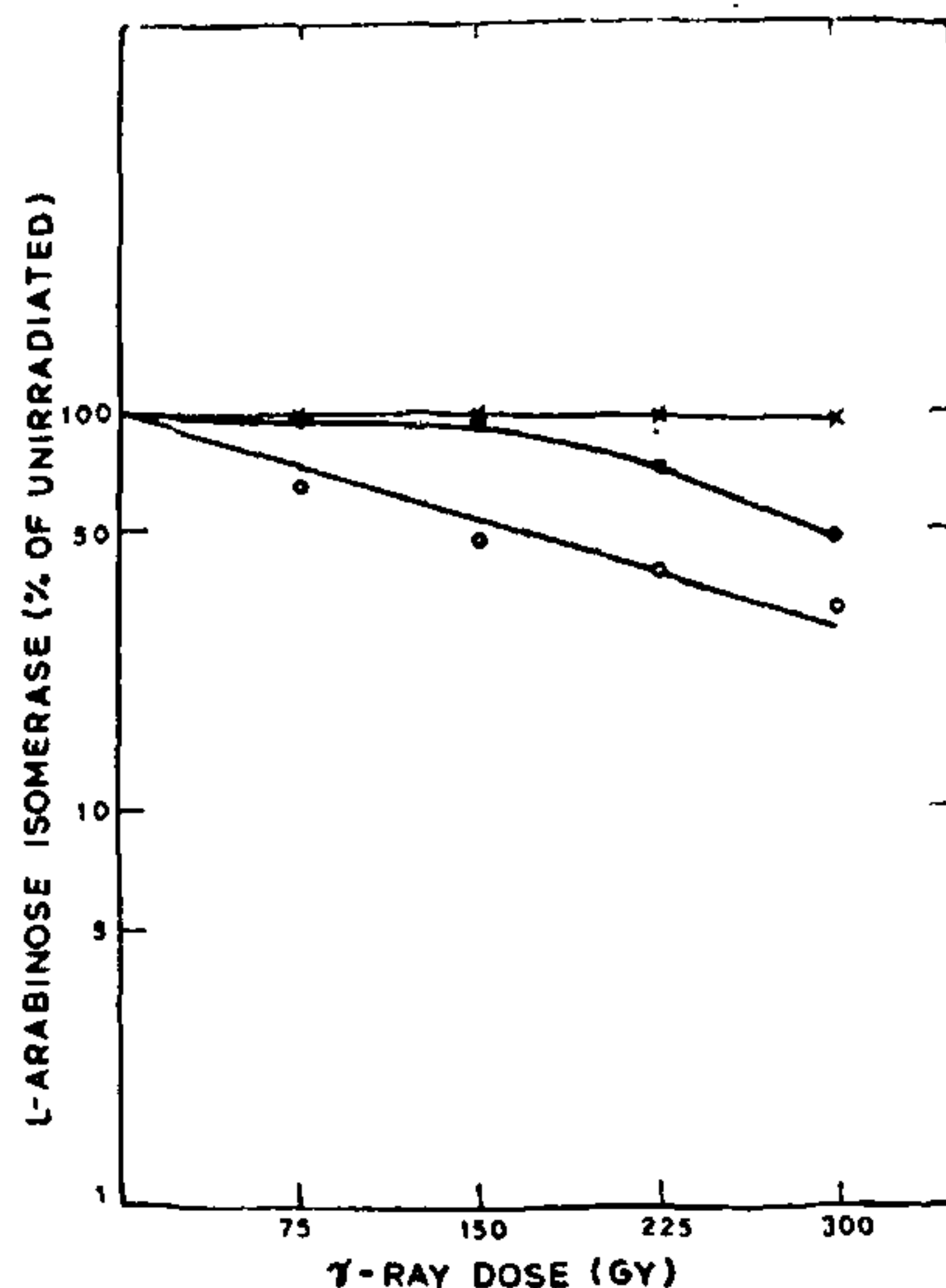

Figure 1. Effect of separation of cells from γ-irradiated cell suspension and their suspension in fresh medium (×) and effect of suspending unirradiated cells in the supernatant from γ-irradiated cell suspension (●) on the induction of L-arabinose isomerase. Tris–EDTA-treated cells were irradiated with different ^{60}Co γ-ray doses as indicated. The symbol (O) represents L-arabinose isomerase synthesizing capacity of γ-irradiated cell suspension without separating the cells from the suspension. The enzyme induction for 60 min in unirradiated cell suspension has been assumed to be 100%.

arabinose isomerase in the unirradiated cells. These results suggested that γ-irradiation of cell suspension caused production of some material(s) which was responsible for inhibiting enzyme induction. The inhibitory material(s) was produced by irradiation due to the action of γ-rays on the cells and not on the suspending medium because L-arabinose isomerase synthesizing capacity of cells were not at all affected when they were suspended in growth medium (without cells) irradiated with γ-rays (data not presented). Thus it appears that radiation-damaged bacteria produced some catabolites which were released in the suspending *medium and were responsible for the inhibition of L-arabinose isomerase synthesizing capacity.*

Since the inhibition of L-arabinose isomerase synthesizing capacity of *E. coli* B/r cells by γ-irradiation with high γ-ray doses (180 Gy and above) was shown earlier to be enhanced by post-irradiation incubation of the cell suspension[1] it was of interest to find out the effect of incubation of γ-irradiated cells following their separation by centrifugation from γ-irradiated cell suspension and resuspension in fresh growth medium.

Such incubation led to an exponential decrease in the enzyme synthesizing capacity when the dose was 180 Gy and above, whereas at a lower dose (60 Gy) the capacity remained unaffected (Figure 2). To test whether incubation of γ-irradiated cells suspended in fresh growth medium led to the release of some inhibitory materials thereby causing the decreased enzyme induction, the supernatant collected following the incubation was added to unirradiated cells and their enzyme synthesizing capacity was determined. The supernatant from irradiated cells incubated following their suspension in fresh growth medium did not cause any change in L-arabinose isomerase synthesizing capacity (Table 1). Thus, the release of inhibitory material(s) was not the cause of the decreased inducibility of the irradiated cells following their incubation in fresh growth medium.

The inhibitory effect of the supernatant obtained from γ-irradiated cell suspension on L-arabinose isomerase synthesizing capacity of unirradiated cells was withdrawn following post-irradiation incubation of the irradiated cell suspension (Figure 3). This is concomitant with the inability of the irradiated cells to
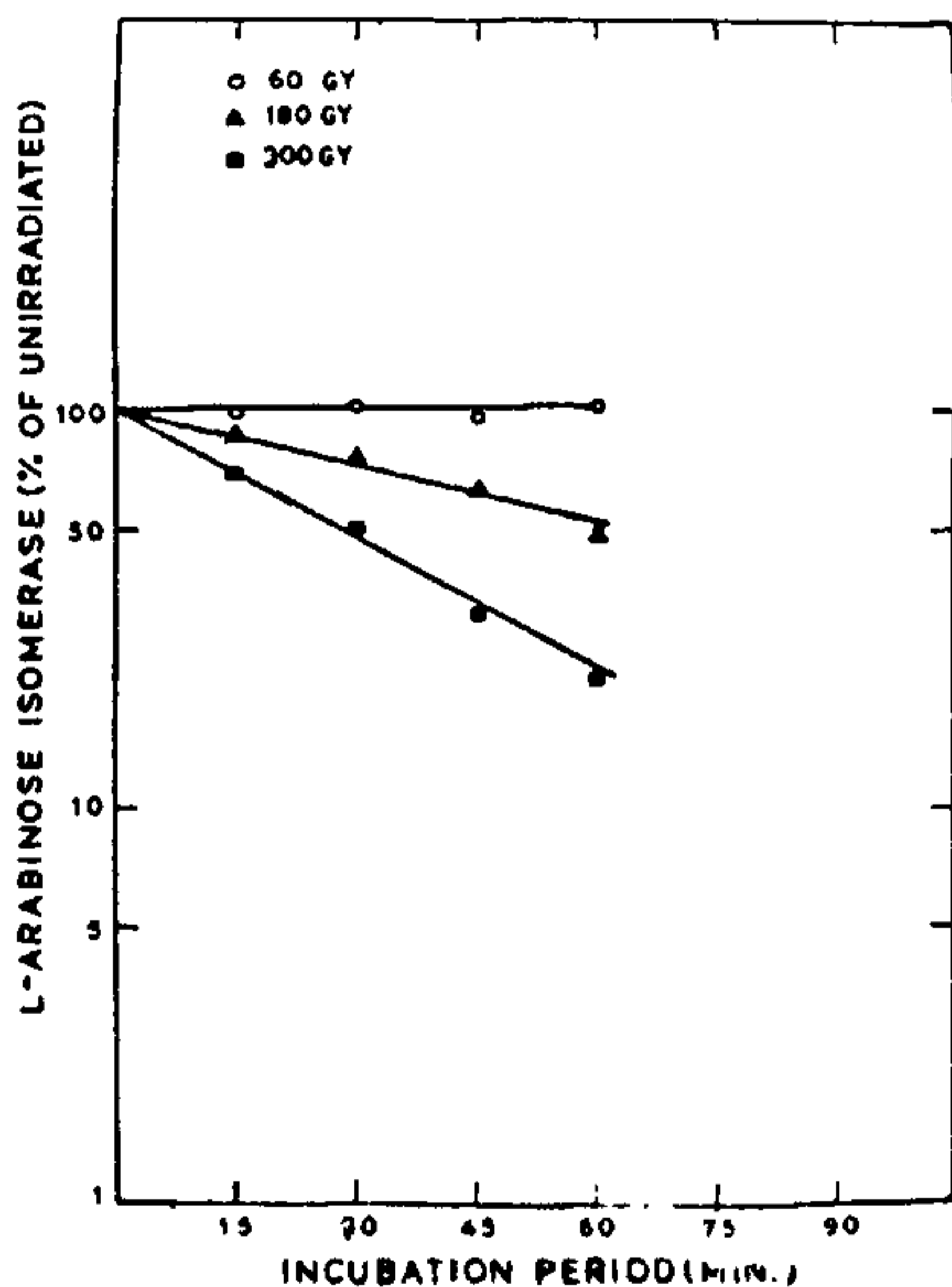
Table 1. Effect of supernatant from cell suspension obtained following incubation of γ-irradiated cells suspended in fresh growth medium on L-arabinose isomerase induction in unirradiated cells

Induction in	L-arabinose isomerase (Units/ml)
Unirradiated cell suspension	36
Unirradiated cells suspended in supernatant from irradiated cell suspension	21
Cells obtained from irradiated cell suspension suspended in fresh growth medium	38.5
Unirradiated cells suspended in supernatant from γ-irradiated cells suspended in fresh growth medium and incubated	
for 30 min.	39
for 60 min.	37

Immediately after γ-irradiation with a dose of 300 Gy, the cell suspension was centrifuged and the cell pellet was suspended in a fresh medium. Induction was carried out for 60 min.
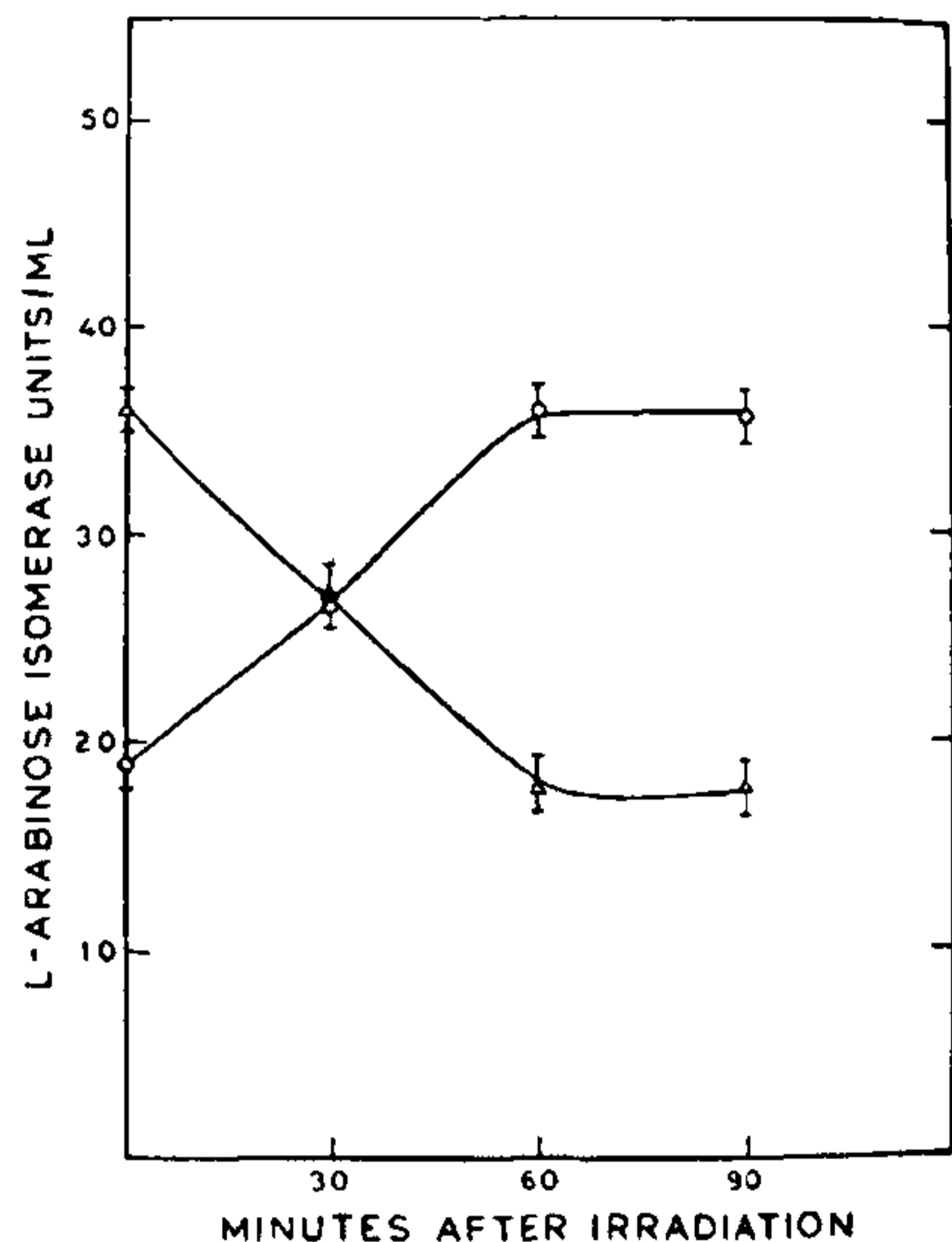
Figure 2. Effect of incubation of γ-irradiated cells suspended in fresh growth medium on their ability to synthesize L-arabinose isomerase. Immediately after irradiation with different γ-ray doses, as indicated, cell suspension was centrifuged, and the pelleted cells were resuspended in fresh growth medium. This suspension was incubated at 37°C with constant shaking. After varying intervals (min), as indicated, L-arabinose was added and induction was carried out for 60 min

Figure 3. L-Arabinose isomerase induction in unirradiated cells suspended in the supernatant obtained from γ-irradiated cell suspension following post-irradiation incubation (○) and that in pelleted cells obtained from post-irradiated incubated cell suspension, suspended in fresh growth medium (△). γ-Irradiation was done at a dose of 300 Gy. At varying intervals of time following post-irradiation incubation, γ-irradiated cell suspension was centrifuged. These pelleted cells were resuspended in fresh growth medium and the supernatant was used to suspend unirradiated cells. Induction was carried out for 60 min. Error bars represent the standard deviation calculated from four independent experiments.

withdraw the inhibition of enzyme induction by their suspension in fresh growth medium (Figure 3). It appears that the catabolites, produced by irradiated cells and responsible for causing γ-ray induced

catabolite repression, were degraded following post-irradiation incubation of the cell suspension.

Cyclic AMP could reverse the inhibition of enzyme induction in unirradiated cells produced by supernatant from irradiated cell suspension obtained immediately after irradiation (Figure 4), indicating that this inhibition is caused by catabolite repression. The effect of cyclic AMP became much less with supernatant from post-irradiated incubated cells, showing that L-arabinose isomerase synthesizing system was released from the catabolite repression by inhibitory material(s) following post-irradiation incubation. This effect is similar to the effect of incubation of UV-irradiated cells which also leads to the release of catabolite repression of the system synthesizing β-galactosidase[7] and L-arabinose isomerase[8].

It is clear from the present study that there are two distinct types of effect of γ-rays on the enzyme synthesizing system. The first is the cyclic AMP mediated γ-ray induced catabolite repression[1,2] which is similar to the catabolite repression induced by UV light[5,8,9]. Catabolite repression induced by both UV- and γ-irradiation appears to be due to production of catabolites by damaged bacteria. However, the catabolites are released from the γ-irradiated cells into the suspending medium whereas they are accumulated in the cells following UV irradiation and are not released into the growth medium (data not presented). The second type of effect of γ-rays on the L-arabinose isomerase synthesizing system is due to a process other than catabolite repression. While the first type of effect is expressed immediately after irradiation, the expression of the second type of effect occurs only after incubation of the irradiated cells.

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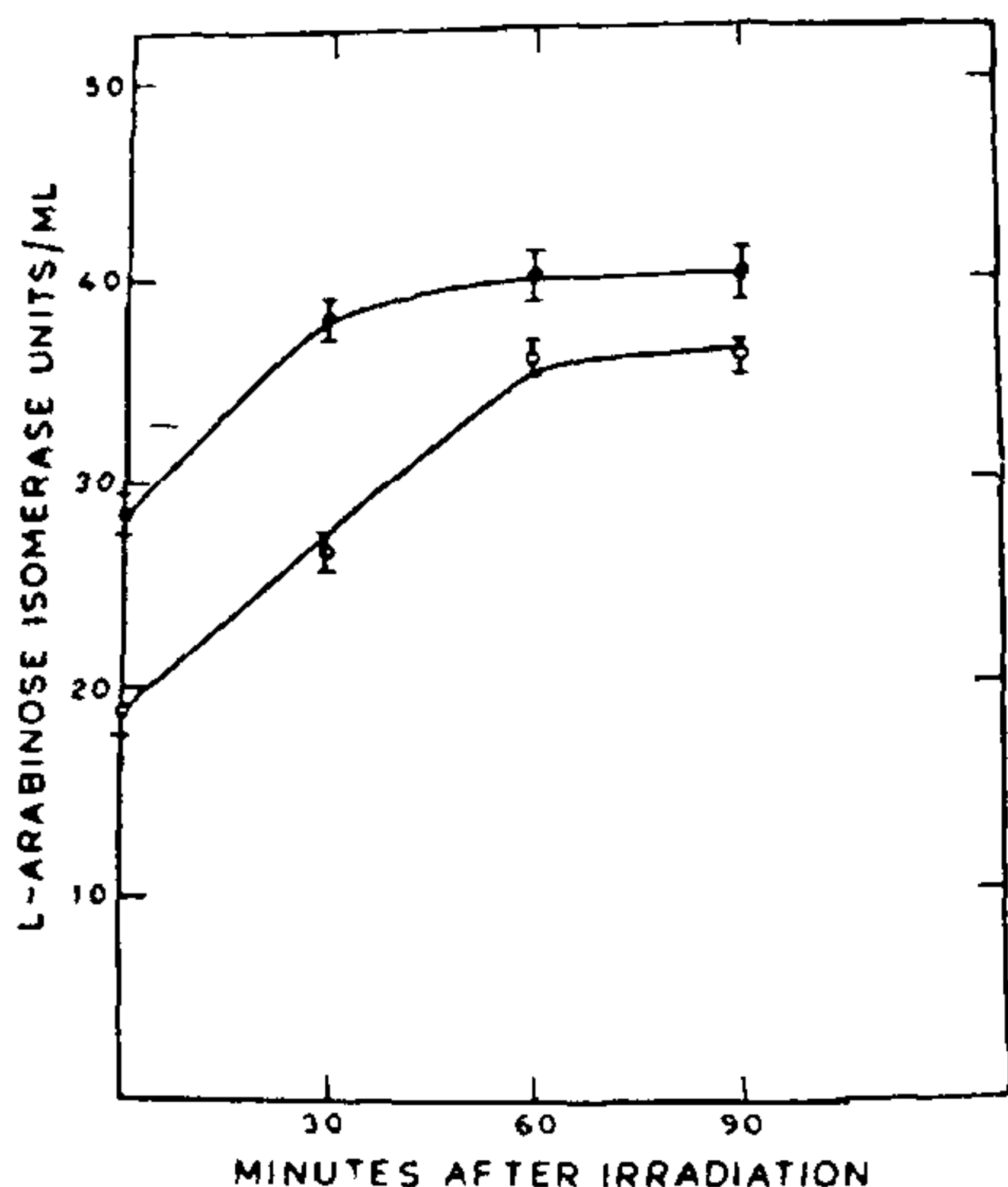9. Bhattacharya, A. K., Biochim. Biophys. Acta, 1974, 354, 71–74.

Figure 4. Effect of cyclic AMP on L-arabinose isomerase induction in unirradiated cells suspended in the supernatant obtained from γ-irradiated cell suspension following post-irradiation incubation (●). Incubation was done for varying periods as indicated. The symbol (○) represents the values obtained without cyclic AMP. The dose of irradiation was 300 Gy and cyclic AMP was used at a final concentration of 5×10^{-3} M. Error bars represent the standard deviation calculated from four independent experiments.

Analysis and characterization of Charminar lime plaster

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The analysis and characterization of ancient lime mortars are important both from historical and restoration points of view. In the present study, lime plasters of Charminar have been analysed by atomic absorption as well as classical chemical techniques and the analytical data have been used to evaluate the mineralogical composition of the plasters by further simplifying the three equation system suggested by Dupas. The result gives more realistic interpretation of the nature of mortar and indicates the technology adopted in the preparation of Charminar plasters.

ANALYSIS and characterization of lime plasters of different periods are important not only from archaeological but also from restoration point of view. For replastering of damaged plasters, it is essential to use plasters of composition bracketing the composition of the analysed plasters to avoid formation of cracks at a later stage. Besides, strength and durability of the plasters can be adduced from the characterization of its raw materials.

Studies indicate that ancient mortars have been