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BASIC ASPECTS OF RADIATION ACTION ON MICROORGANISMS

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The writing of a termination report on this project cannot be justified in scientific terms since it is proposed (proposal submitted) to continue the work at Duke University. Thus it is actually continuing work and not really at a termination stage. To appreciate the situation as of July 1, 1977, only five months ago, the progress report then submitted (COO-2362-26) can be consulted. In what follows a brief account of findings made since that time will be given. This, together with the July 1 progress report and reprints of an article published recently (Pollard & Snipes, Photochem. Photobiol. 26, 545, 1977), constitutes the termination report.

I. THE EFFECT OF ANTIPAIN ON THE INDUCED REPAIR PHENOMENA OBSERVED IN OUR LABORATORY

It was mentioned in the July 1 report that very interesting work had been done on a protease inhibitor called antipain. It seemed to suggest that a good many of the phenomena that are associated with induced repair could have as one stage in their action a protease which was operating on some part of the cell.

In the report by Meyn, Rossman & Troll (PNAS 74, 1152, 1977) they mentioned that quite possibly the inducible inhibitor of postradiation degradation might be found to be insensitive to the action of antipain, in other words not to require the operation of a protease in its system. Because this is of great interest to us, we requested 100 mg of antipain. This was duly delivered to us and we tried it out, with particular intent to see whether its action on induced inhibition is considerable or negligible. We found it quite difficult to demonstrate any effect of antipain on the various manifestations of induction with which we have been working. It was very easy to show that antipain most effectively inhibits trypsin. However, we found that in moderate concentrations there seemed to be no effect on induced radioresistance, no effect on the

induction of lambda in K12 λ , and no effect on the inhibition of DNA degradation.

However, on carefully consulting the original paper, it was observed that unless the medium is particularly lean, and especially unless the carbon source is glycerol, no great effect is observed on the induction of lambda. Accordingly, we changed our method to use higher concentrations of antipain and much leaner medium, and we began to observe what were to us relatively small effects. Such effects we would not normally report as definite, but since they did follow a uniform pattern we are reproducing the data on which we are basing a statement that there is some positive effect of antipain in our work. The first of the data are shown in Figure 1 and concern the addition of antipain to cells that had received a pretreatment of 4.8 J/m² of ultra-violet light (UV). It can be seen that this treatment causes the cells to become more resistant to gamma radiation, and this is indicated by the diminished downward slope as shown in the figure. As the result of the presence of antipain this same dose produced less effect and the cells were slightly less radioresistant than in its absence. The difference between the slopes of these two lines we believe to be just barely significant, and our estimate is that there has been perhaps a 10% reduction in the amount of radioresistance that has been conferred by the pretreatment with UV. This is a marginally definite result.

The second observation concerns the induction of lambda in K12 λ . The technique used is that employed by Coetzec & Pollard and previously reported (Photochem. Photobiol. 22, 29, 1975). This involves growing cells overnight on lactose so that they are fully induced for β -galactosidase. The cells are then exposed to ONPG, the substrate for this enzyme, and because the intact cell wall and cell membrane are between the enzyme and the substrate no reaction can occur. However, when lambda is induced in the cells there

Figure 1. Cells of AB 1157 are either given UV treatment, with and without antipain, followed by incubation for 40 minutes and further treatment with rifampin to block further protein synthesis, or else just rifampin. They are then exposed to gamma rays and the survival ratio measured.

is generation of endolysin which breaks down the cell wall and permits the two to come together, thus producing considerable yellow color which can be estimated quantitatively in the spectrophotometer. The results of giving various doses of UV to cells and then following the development of color in this reaction, color which indicates the breakdown of the cell wall by the induction of lambda, are shown in Figure 2. In Figure 3 there is a summary of the relative proportion of induced cells estimated from the amount of color produced; included in the figure are the two cases where antipain at a final concentration of 1 mM was used to attempt to influence the process. These two points are marked by circles and it can be seen that there is a small depressing effect of the antipain but that this is not really very considerable.

The findings on the inhibition of DNA degradation are shown in Figure 4. The normal amount of depression of degradation produced by a preliminary dose of UV is seen, and if to this same culture is added 1 mM antipain there is somewhat less DNA degradation. The difference once again amounts to about 10%. Ordinarily this would not be considered important, but in view of the findings of others and our own experience perhaps we should say that this is significant. In any event, there is as much effect on inhibition of DNA degradation as there is on the induction of lambda or the production of radioresistance.

In order to check whether our technique was in some way deficient with regard to inhibition, we looked at a secondary method of inhibiting which has also been reported by the group at New York University (Meyn et al., op. cit.) - the use of arsenite. We found that arsenite had a quite clear effect on all three of the phenomena of our study - radioresistance, induction of lambda, and the inhibition of DNA degradation. The effect is reversible. When the arsenite is removed the looked-for effects return. Therefore, we

Figure 2. Observation of the induction of K12 by UV, by measuring the cell leakiness due to the formation of enolysin following induction. The cells are grown on lactose to induce β -galactosidase and are then exposed to ONPG, a substrate for the enzyme which generates color when it is split by the enzyme. The amount of color developed is a measure of the amount of induction. Antipain, at 1.2mM is added in the two cases shown. There is a marginal effect.

Figure 3. The percent of maximum induction for different doses of ultraviolet light. The solid line is observed by the endolysin method; the dashed by the plaque assay method. The effect of antipain can be judged by the open circles, which suggest a slight reduction at 18.7 J/m². The effect is marginal at best.

Figure 4. The modification of DNA degradation inhibition by antipain. The pre-treatment with UV induces the inhibitor of post irradiation DNA degradation and there is appreciably less degradation. Giving antipain as well as the ultraviolet treatment slightly reduces the amount of inhibition.

conclude that there is, in all probability, a complication about getting the small polypeptide antipain into the cell. Undoubtedly growth conditions may affect this. Using growth conditions which have been reported to be favorable we were able to show a marginal effect of antipain, not of a size that we are anxious to report, but nevertheless we believe it to be there. We can say that if this marginal effect is real, then it is at least as evident for the case of inhibition of DNA degradation as it is for the other phenomena.

II. Micrococcus luteus

In our attempt to see how general is the phenomenon of induced repair, we grew up cultures of M. luteus. This microorganism is studied considerably in connection with UV excision repair, and we examined it to see whether there was a change in gamma-ray sensitivity introduced by pretreatment with UV. It proved necessary to develop the technique of sonicating the cultures in order to break up the clumps of cells which form, but when this was done it was quite possible to plate M. luteus, and the kind of data obtained are shown in Figure 5. It can be seen that a small predose of UV, which corresponds to a point on the shoulder of the survival curve for the action of UV, produces not only no protection against the X-rays, but actually sensitizes slightly. We believe this is evidence that in this microorganism one manifestation of induced repair is not present.

III. "Frozen UV"

The observations reported earlier by M. Rader on the action of "frozen UV" were followed up to study the induction of lambda. To recapitulate slightly, it was found that giving UV at temperatures of Dry Ice and methanol caused very good induction of the induced repair system but had somewhat different properties with regard to mutagenesis. We used the same system that was described previously for the observation of the induction of K12 λ

Figure 5. Failure to observe UV-induced radioresistance in *Micrococcoccus luteus*. A sonicated culture was exposed to UV as indicated, incubated for 70 minutes to allow expression of any radioresistance, given chloramphenicol to block further induction by gamma radiation, and then exposed to varying doses of gamma radiation in a ⁶⁰Co source. There is no increased resistance, but rather a slight increase in sensitivity.

and found, first, that the effect of freezing alone, or rather combined freezing and thawing, is to produce a considerable breakage of the cells because they show the color reaction, corresponding to damage in the cell wall, right from the very beginning without incubation. The effect of further incubation is to show that lambda has been induced because more color does show, but there is much less color than would be observed with the same amount of UV in the melted condition.

This has been followed up by an examination of the actual induction of lambda as observed by the plaque method. The findings surprised us because whereas the non-lysogens survived the process of freezing and thawing to an extent of greater than 50%, this was not true in the case of the lambda lysogen. We found that only something like 3-5% of the cells survived and some of this was traced to the method of thawing. With care it was possible to retain as many as 15% of the cells by quicker thawing in more warmth, but this is not really a large enough number for firm conclusions. We can say that as regards the cells that survived freezing and thawing, the action of UV light in this condition is just as good at inducing lambda as is the normal application of UV light.

CONCLUSION

As has been said, this is not in any true scientific sense a termination report because the work is definitely continuing. The overall project at Penn State University has run for a long time and in order to make a summary of all the findings reference would have to be made to a number of papers and review articles; it does not really make sense to do this in a continuing research project. It is possible to mention the two stresses which have been put on the overall direction of this project. The first stress, naturally, has been to generate solid and non-controversial scientific data which will

stand up to repetition by others and will add to the facts that are known in the field. Thus we have emphasized thoroughness in the work. The findings have been reported and the list of papers published is long and considerable; very little indeed of the work has been challenged at all.

The second direction of the work concerns the training of personnel. In the university setting this is an inescapable and irresistible fact, and rightly so. As a result of this work a moderate number of students have been trained to the M.S. and Ph.D. levels, and some training of postdoctorals has also occurred. Of the Ph.D.s trained, nine are actively pursuing radiation studies. Several have become quite solid in the field, notably J. D. Chapman, C. E. Hildebrand, and P. M. Achey. This group is still quite young, and it appears probable that they will show individual strength in much the same way that the earlier group trained at Yale have shown it, so that there will be significant contributors such as Hutchinson, Fluke, Till, Whitmore and Woese, who are representative of the Yale-trained group.

It is hoped in continuing the work in collaboration with Dr. D. Fluke that some more of this training process can be initiated. It is not quite so easy to do as it was and perhaps not so valued as formerly by the granting agencies, but in my own judgment it is still very vital to our nation.

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