MOLECULAR AND CELLULAR REPAIR PROCESSES

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The acceleration in the investigation and understanding of molecular repair mechanisms, particularly from the enzymatic viewpoint, and growing appreciation of the nature of cellular repair processes in response to acute and chronic injury from a variety of environmental agents, including chemical, ultraviolet and ionizing radiation, make timely the correlation of those disciplines concerned with mechanisms of repair processes. The knowledge may be applied to such matters as the induction of selective injury to malignant cells and the establishment of standards of maximum permissible levels of exposure to toxic, mutagenic and carcinogenic agents. Under the sponsorship of Miles Laboratories, Inc., chaired by Professors Roland F. Beers, Jr. and Roger M. Herriott, of The Johns Hopkins University School of Hygiene and Public Health, this Symposium was arranged and the record of the proceedings constitutes Supplement Number 1 of The Johns Hopkins Medical Journal.

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INTRODUCTION

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Among the properties assigned to living organisms are the capacities to grow differentially and to replicate. The former frequently leads to overspecialization with its attendant high risks against survival of the species; the latter, depending upon its relative efficiency, compensates for those risks.

There is a third property of life to which we are addressing ourselves during the next two days, namely the capacity for self-repair. It is a subject which is as broad as the life sciences themselves and can be said to be the basis for most biomedical research motivated by clinical needs. The capacity of an organism to repair by replacement or restoration of a damaged component is vital to its functional survival but the success of the repair process is dependent upon the magnitude and duration of the stress load and resultant damage.

We are living in an era noted for its concern for the biological consequences of environmental stresses which range from ionizing radiation to chemical carcinogens to sonic radiation. Every conceivable product of our technological civilization is coming under close scrutiny for its potential damaging effects on living organisms. The emergence of a no-risk criterion of acceptability of any product, as embodied in the Delaney amendment to the Food and Drug Act and expressed by many crusaders for the public welfare today, reflects an underestimate of the capacity of living organisms to cope with stresses.

Indeed, living organisms survive and evolve precisely because of and not in spite of stresses imposed upon them. At our stage of ignorance about biological repair processes it is probably of equal folly to claim that some stresses, such as radiation, are permanently damaging at all dose levels or to make the counterclaim that all stresses are beneficial at proper dose levels. Depending upon the circumstances both claims may be right or wrong. Critical for any such assessment is a knowledge of the role of repair processes.

The subjects that have been chosen for this Symposium are concerned with repair processes at the molecular (or enzymatic) level and at the cellular (or subcellular) level. I would hope that during the discussions the speakers will have time to reflect and comment on several issues relevant to the initiation and consequences of repair processes.

For example, consider the relationships between dose response and repair processes. To what extent are threshold or pseudothreshold levels of toxic agents a function of repair? What factors, such as time or post exposure conditions, modify repair processes? What consideration should be given to repair processes by public officials in reaching decisions about maximum permissible levels of exposure.

Another issue is the experimental rationale for detecting threshold phenomena. The extensive and extremely tedious methods of evaluation employed by the Russells and Bentley Glass, who utilized mice and Drosophila, respectively, to study low levels of radiation, have reached a point of costly diminishing returns. Where threshold phenomena are a function of repair processes, it may be important to consider an experimental model in which the repair processes are blocked. What information can we gleam from such a model?

This problem of tolerance levels is equally important in evaluating the biological effects of drugs, food additives, pesticides and other exotic chemicals. The massive long-term screening programs required for approval of new and old drugs suffers from the inherent impossibility of proving a negative. The politically expedient solution implicit in the Delaney amendment reflects that imponderable problem. History indicates that the search for absolutes in safety and in risks and their implementation through legal processes is as difficult a task as any attempted by a civilization expecting to govern itself on the basis of absolutes.

The validity of extrapolating to man results obtained with bacteria, insects or lower mammals, when it is very likely that many of the mechanisms and efficiencies of repair processes differ widely from species to species and possibly from strain to strain of a given species, is questioned with increasing frequency.

A question which is just beginning to gain attention is the role of repair processes in maintaining genetic stability. The popularity and beauty of the Watson-Crick model of DNA lies in part in its stability because of its structural configuration, a stability that persists during the replication process. If repair processes are important in maintaining genetic stability, then the evolutionary development of repair mechanism becomes of critical importance.

Permanent mutation in an organism may depend upon the absence of an effective repair process that can reverse the mutation, although a repair process might well perpetuate the mutation through its incorporation into the functioning DNA. The role of repair processes and their manipulation in genetic "engineering" are parameters that must be included in evaluating any feasible objective in this rapidly developing field of applied genetics. Reverse mutations, translocations and hybridization are among some of the phenomena which are influenced by repair processes.

Finally, there remains the important relationship between aging and repair processes. If aging results in part from a failure of repair mechanisms, this failure can occur either because the lesions produced with age are not reparable by existing systems of repair or because the systems themselves may become deficient. Since aging is also a function of stress on the organism, clearly the apparent accelerated aging process brought about by such stressful agents as radiation and toxic compounds, hormonal and genetic factors, and diet must be examined in terms of their relationship to repair processes.

Although this Symposium is only indirectly concerned with the specific biochemical lesions susceptible to repair, it is obvious that an understanding of the mechanisms of repair must include a knowledge of the lesions involved. The meaning of

"sensitivity" of an organism to a particular stress is a function of both parameters, although until recently repair mechanisms have not been consistently identified as such. It is quite clear that some "repair processes" may lead to severe or lethal abnormalities, depending upon the kind of lesion produced. Thus, repair processes should not be judged to be unusually beneficial for the survival of the individual organism or of the species. Like stress, repair mechanisms of the cell may be a two-edged sword.

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Part I

ENZYMATIC DARK REPAIR PROCESSES

MAMMALIAN DEOXYRIBONUCLEASES ACTING ON DAMAGED DNA

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Both microorganisms and mammalian cells have the ability to remove various types of damaged nucleotide residues from their DNA. One mechanism of repair apparently involves the consecutive action of at least four different enzymes: an endonuclease that recognizes a particular type of DNA damage and catalyzes the formation of a single-strand break close to the damaged nucleotide(s), an exonuclease that excises this residue and a number of additional nucleotides, a DNA polymerase responsible for repair replication and a DNA ligase that rejoins the remaining strand interruption. Enzymes of all four types have been found in microorganisms, and their function in DNA repair has been confirmed by the isolation of radiation-sensitive mutant strains with defective enzymes (1-3).

As mammalian cells are also known to respond to radiation damage by DNA excision, repair replication and joining of interrupted DNA strands (4-6), it appears likely that similar repair enzymes are present in higher organisms. A DNA ligase (7) and a DNA polymerase (8) have in fact been found in the nucleoplasm of mammalian cells.[2] Because of the lack of radiation sensitive mutant cell types, it is not possible to prove directly whether these mammalian enzymes function in DNA repair in vivo. However, as the purified mammalian DNA ligase and DNA polymerase have biochemical properties similar to the corresponding microbial enzymes, it is a reasonable working hypothesis to assume that the mammalian enzymes are indeed involved in DNA repair. This indirect approach of comparing the biochemical properties of mammalian enzymes potentially functioning in DNA repair with microbial enzymes of known function has now been extended to several nucleases.

ENZYMATIC EXCISION OF PYRIMIDINE DIMERS

Two exonucleases, referred to as DNase III and DNase IV, have been found in the nucleoplasm of rabbit cells and have been partly purified (9,10). Both enzymes have an alkaline pH optimum, require Mg^{++} and lack DNA polymerase activity in vitro. They release 5′-mononucleotides as the main degradation product, but also liberate a minor part (15% - 20%) of the products in the form of small oligonucleotides (9,11). The DNase III attacks at 3′-ends and degrades denatured DNA and single-stranded polydeoxynucleotides four times more rapidly than double-stranded substrates. In contrast, DNase IV attacks at 5′-ends (with either a P- or an OH- end group) and degrades the

[1]The author's work was supported by the Swedish Natural Research Council and the Swedish Cancer Society.

[2]Proteins in the "nucleoplasm" or "nuclear sap" are not firmly associated with the chromatin and leach out on extraction of cell nuclei with an isotonic salt solution.

double-stranded synthetic polydeoxynucleotide poly (d(A-T)) much more rapidly than single-stranded polydeoxynucleotides or native or denatured DNA from natural sources. The relative ability of the enzymes to degrade UV-irradiated DNA is shown in Figure 1. DNase IV was not detectably inhibited by the presence of pyrimidine dimers in native DNA exposed to high doses of UV irradiation (Fig 1A) though a small inhibitory effect due to irradiation has been noted of the relatively much more rapid degradation of poly (dA · dT) (11). The unusual ability of this exonuclease to hydrolyze effectively

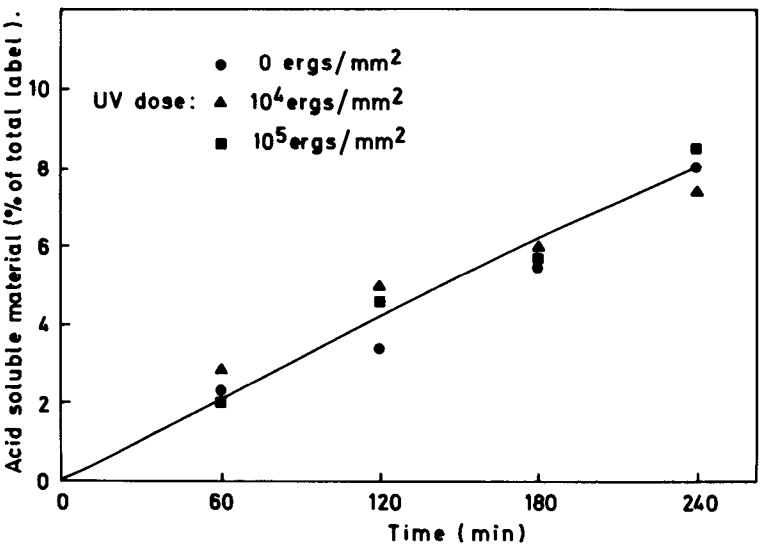

Fig 1A. Enzymatic degradation of unirradiated- and UV-irradiated native E. coli [^{32}P] DNA at 37°. Hydrolysis by DNase IV. The DNA (20 μM in nucleotide equivalents, mol wt 10^7) was incubated with the enzyme (2 units/ml) in 0.07 M Tris-HCl, pH 8.3, 0.004 M $MgCl_2$, 0.001 M EDTA, 0.001 M 2-mercaptoethanol, 0.01% bovine serum albumin. Aliquots were removed at times indicated, chilled, precipitated with an equal volume of 0.8 M $HClO_4$ and the amount of acid-soluble radioactive material was determined.

UV-irradiated substrates suggests that the enzyme may be responsible for the excision of damaged residues during DNA repair in vivo. In contrast, DNase III was strongly inhibited by the presence of pyrimidine dimers in DNA (Fig 1B). It, therefore, seems unlikely that the in vivo function of the latter enzyme involves excision of radiation products from DNA.

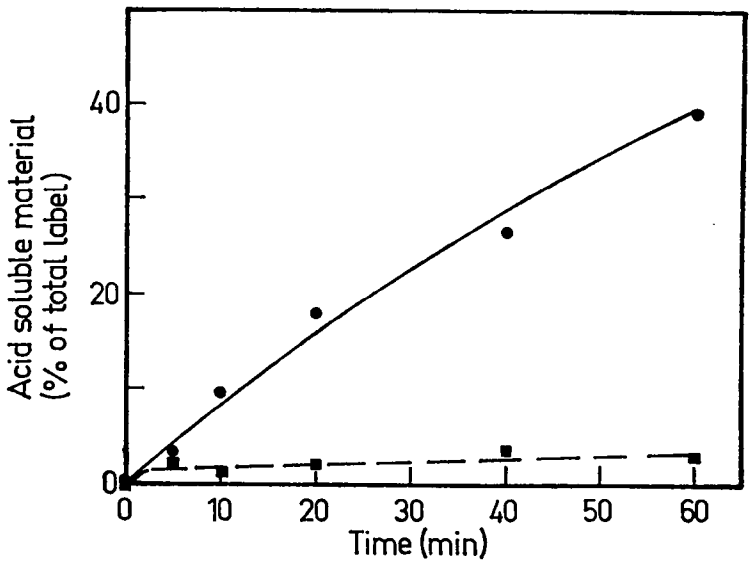

Fig 1B. Enzymatic degradation of unirradiated- and UV-irradiated native E. coli [^{32}P] DNA at 37°. Hydrolysis by DNase III. The DNA (20 μM) was incubated with the enzyme (10 units/ml) in 0.05 M Tris-HCl, pH 8.5, 0.005 M $MgCl_2$, 0.001 M EDTA, 0.01 M 2-mercaptoethanol, 0.01% bovine serum albumin.

●——————● non-irradiated substrate

■— — — —■ irradiated substrate (exposed to 4 x 10^3 ergs/mm^2).

DNase IV releases pyrimidine dimers from DNA as parts of small oligonucleotides (11). The enzyme has two properties in common with exonuclease activities from bacteria that appear to be involved in DNA repair: the rate of hydrolysis is not markedly reduced when pyrimidine dimers are present in the substrate, and the enzyme degrades its substrate in the 5′→3′-direction.

Of the bacterial activities of this type, the 5′→3′-nuclease function of E. coli DNA polymerase (12) shows a strong preference for double-stranded substrates, while the M. luteus UV exonuclease prefers single-stranded DNA (13). These observations indicate that the relative rates of attack on double-stranded vs single-stranded substrates may be of little importance for exonucleases involved in DNA repair. The mammalian DNase IV, purified from rabbit tissues, was originally thought to be specific for double-stranded substrates (10), but recently also has been found to degrade denatured DNA at a slow rate. Moreover, an apparently very similar enzyme has been obtained from rat tissue (lungs) by