AGING AND DNA REPAIR CAPABILITY

by

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1. INTRODUCTION

The aging process is best defined as a general loss in biological competence for both the individual cell and the organism as a whole. At the cellular level this loss is expressed as decreasing replicative ability in proliferating cells and decreasing functional activity in post-mitotic cells (Little, 1976). For the organism, the aging process expresses itself as decreased viability and increased vulnerability to the normal forces of mortality (Goldstein, 1971a).

Current aging theories can be largely divided into two categories: those based on genetic programming and those based on error accumulation (Hayflick, 1975; Medvedev, 1976; Cutler, 1976c). The former theories assume that aging is actually a continuation of growth, differentiation and morphogenesis (i.e. that aging is genetically determined). The latter theories assume that the primary aging event is stochastic (i.e. not genetically determined but governed by chance) and expresses itself by accumulation of errors in DNA, RNA, and/or protein.

Fundamental to most aging theories is the importance of the maintenance of DNA integrity and the possible primary role of DNA repair processes (Samis, 1966; Alexander, 1967; Smith, 1976; Trosko and Hart, 1976). At least one aging model is, in fact, based on the possible differential repair of mutational damage in transcribed and nontranscribed DNA (Yielding, 1974). DNA repair processes, by monitoring potential mutational events, clearly represent a major homeostatic mechanism at the cellular level. Aging, then, could be the result of a declining repair capability which would permit the rapid accumulation of deleterious events at the DNA level. Alternatively, the variation in aging rates between different animal species might be due to intrinsic differences in the efficiencies of their DNA repair systems.

Both alternatives assume that aging is a direct consequence of unrepaired mutational events. The former possibility predicts that unrepaired events should rise precipitously near the end of an organism's lifespan as DNA repair capability becomes increasingly inefficient. The latter possibility predicts the presence of a direct age-related increase in unrepaired DNA lesions which should be proportional to the length of the normal lifespan. If one or both of these possibilities exists, then predictably,

→ (1) an age-related increase in unrepaired DNA lesions, whether linear or exponential, should occur; (2) genetic syndromes which express features of premature or accelerated aging should express a DNA repair deficiency component and vice-versa; (3) the rate of the aging process itself should be subject to modification by changes in the environmental levels of DNA damaging agents; and (4) species with different lifespans could be expected to have demonstrable differences in DNA repair capabilities and/or DNA repair capability should decline with age.

The following review will examine each of these predictions, in vitro as well as in vivo, in an attempt to assess the relationship between normal aging and DNA repair capability.

2. DNA REPAIR PROCESSES

Investigations which concern themselves with DNA repair in eukaryote cells are not far removed from their infancy. Slightly more than ten years have passed since the first indication of an active DNA repair process in mammalian cells (Rasmussen and Painter, 1964). In that short time, a bewildering array of findings and conclusions have been published, and the reader is directed to some extensive reviews on this subject (Hart and Trosko, 1976; Little and Williams, 1976; Hamawalt and Setlow, 1975). What is apparent is the existence of at least four types of DNA repair--strand break rejoining, excision repair, postreplication repair, and photoreactivation. The first two

repair systems are thought to involve a large number of subcomponents, each of which may be specific for the repair of a certain type of DNA lesion. However, in some instances the same DNA lesion can be repaired through the activity of more than one DNA repair process. Consequently, when speaking of the DNA repair capability of a cell, a tissue, or an organism, we are in fact speaking of the sum total of all repair processes present and the interaction between these systems to monitor and repair potential DNA lesions. The following description of these repair processes and the means by which they are assayed is only meant to serve as a basis for evaluating the possible relationship between aging and DNA repair and not as an extensive summary of the molecular mechanisms involved in DNA repair.

2.1. Strand Breakage Rejoining

DNA strand breakage is a direct consequence of exposure of cells to ionizing radiation or to a few so-called radiomimetic compounds (e.g. methylmethanesulfonate). It is not known whether base and sugar damage accompanies (or precedes) strand breakage, nor is it known whether base release sometimes or always accompanies break formation (Painter, 1974). What is known is that repair of this type of lesion(s) is rapid and involves limited exonuclease activity (Dugle and Gillespie, 1975; Hart and Trosko, 1976).

2.2. Excision Repair

Excision repair is basically a process by which the cell repairs DNA base damage. It is probably the most extensively examined repair system to date and seemingly the most complex (Hart and Trosko, 1976). In its most simple form, excision repair involves the recognition of a base damage and the production of a nearby incision by an endonuclease, excision of the damaged base and some adjacent bases by an exonuclease, insertion of new bases to fill the excised region

(repair replication), and a ligase activity to seal the loose ends (Painter, 1974). There exist at least four classes of endonucleases, each of which appears to be specific for the different types of DNA lesions induced by ionizing radiation, UV light, or chemical compounds (Hart and Trosko, 1976).

2.3. Postreplication Repair

Excision repair is not the only DNA system which acts to repair base damage. Another process, known as postreplication repair for its dependency upon semi-conservative DNA replication, also exists (see Hanawalt and Setlow, 1975). Of the known DNA repair systems, postreplication repair is the least understood. Presumably it is a process by which DNA newly synthesized from a defective or damaged template is repaired (Hart and Trosko, 1976). Initially postreplication repair was postulated as a mechanism to fill gaps in newly synthesized DNA occurring opposite base damage in the parental DNA (Lehman, 1972; Buhl *et al.*, 1972). However, this model has not been verified experimentally (Painter, 1974; Meneghini and Hanawalt, 1975). Postreplication repair appears to be coupled to normal semiconservative DNA replication and the two processes might, in fact, share some of the same enzymes (Hart and Trosko, 1976). There is evidence to suggest that this repair process has an inducible caffeine-sensitive component in some permanent cell lines (D'Ambrosio and Setlow, 1976).

2.4. Photoreactivation

Photoreactivation is a repair process specific for UV-induced cyclobutyl pyrimidine dimers. These dimers are repaired by the photoreactivating enzyme in a two-step process. First, the enzyme binds to a dimer-containing DNA region and then in the presence of 300-600 nm light, the cyclobutane ring undergoes photolysis, producing the original two monomere pyrimidines (Sutherland *et al.*, 1974). Only recently has the presence of this enzyme been demonstrated in placental mammalian tissues such as human leukocytes and

murine or human fibroblasts (Sutherland, 1974; Sutherland et al., 1974).

2.5. Assay Systems for DNA Repair

The repair of strand breakage has been generally investigated by techniques based on alkaline sucrose gradient sedimentation (McGrath and Williams, 1966). Not only does this assay system indicate the presence of single-strand breaks but also the occurrence of alkali-labile bonds in DNA. The sensitivity of this assay system depends, to a large extent, on the gentleness of the preparative techniques for isolating large molecular-weight DNA from cells.

Excision repair capability in cells exposed to ionizing radiation, UV light, or mutagenic compounds, has been examined with a number of different techniques--techniques that measure the completion of different steps in the repair process. These techniques include assaying endonuclease activity by the formation of strand breaks (Bacchetti, 1975), the excision step by the release of pyrimidine dimers from DNA (Carrier and Setlow, 1971), and the resynthesis step by repair replication (Cleaver and Painter, 1968) or unscheduled DNA synthesis (UDS) (Rasmussen and Painter, 1964). The technique most commonly used is that of UDS. This assay system involves the incorporation of tritiated thymidine into DNA of cells not actively undergoing semiconservative DNA synthesis, hence the name unscheduled DNA synthesis. Subsequently, this incorporation of a radioactive isotope can be measured on a cell by cell basis by autoradiography (Rasmussen and Painter, 1964) or for the population as a whole using scintillation counting techniques (Evans and Norman, 1968).

Techniques for the analysis of postreplication repair have largely involved measurements of DNA molecular weight by density gradient centrifugation (Painter, 1974) or by alkaline sucrose sedimentation techniques (Lehmann, 1972). Recently, a cytogenetic technique has been developed which might permit the examination of a successful postreplication repair event--sister chromatid

exchanges (Latt, 1973; Kato, 1977). However, the exact nature of sister chromatid exchanges (SCE) and its relationship to DNA repair has not yet been fully resolved and, therefore, SCEs must remain as a potential technique for future experimentation.

Assaying photoreactivation repair depends upon the isolation of the photoreactivating enzyme from tissues and its incubation with UV-exposed DNA and subsequently measuring pyrimidine dimer loss (Sutherland and Chamberlain, 1973).

All of these DNA repair systems can be further assayed in proliferating cells through cytogenetic techniques. Unrepaired DNA lesions manifest themselves at the chromosomal level in metaphase cells as aberrations of one type or another depending upon the agent, the type of DNA lesion induced, and the timing in the cell cycle of the exposure to that agent (Bender et al., 1973a, 1973b, 1974). Consequently, a decrease in DNA repair capability by any of the known DNA repair systems will increase the frequency of observable chromosome aberrations in metaphase cells. This is, of course, the basis for the suggestion that human diseases characterized by increased levels of spontaneous chromosome aberrations involve a deficiency in some DNA repair system. These diseases include such genetic syndromes as Fanconi's anemia and ataxia telangiectasia (German, 1972). What must be remembered with aberration investigations is that in most cases the total capability of the cell to repair DNA lesions is assessed and not the efficiency of one specific repair system.

3. AGE-RELATED OCCURRENCE OF UNREPAIRED DNA LESIONS

One line of evidence for a possible causal relationship between DNA repair processes and aging is based on observations that suggest an age-related increase in the frequency of unrepaired lesions in DNA.

Presumably, a dramatic increase in the frequency of unrepaired DNA lesions in aged cellular populations would indicate the absence of competent DNA repair processes and suggests a correlation between aging, cell death, and a decline in DNA repair capability. Alternatively, the presence of unrepaired lesions, which increase in frequency throughout an animal's life and at a rate inversely proportional to lifespan, gives credence to the suggestion that aging is due to the intrinsic level of DNA repair competence and not to its decline.

3.1. DNA Strand Breakage

While many types of unrepaired lesions may be present in DNA, investigations at the DNA level have been largely concerned with assays restricted to identifying the presence of single or double strand breaks. In one of the earliest investigations, Price et al. (1971) and Modak and Price (1971) assayed the ability of isolated nuclei from brain, heart, and liver tissue of young and aged mice to provide templates for calf thymus DNA polymerase. Since this DNA polymerase requires partially denatured DNA to catalyze the incorporation of deoxyribonucleotide triphosphates and incorporation can be increased by ionizing radiation, this system presumably assays for denatured regions or strand scissions in DNA (Modak and Price, 1971).

These investigators observed an age-related significant increase in mean autoradiographic grains in undenatured nuclei neurons and astrocytes but not of Kupffer's cells or cardiac muscle. Acid-denaturation of aged Kupffer's cells or cardiac muscle produced compared with muscle/significantly increased mean grain counts when/acid-denatured young cells. These results were interpreted to indicate an increase in single-stranded regions of DNA in some types of aged cells, and an increase in acid-sensitive regions in all types of aged cells examined. This increase with age was presumably the result of a decline in DNA repair capability or an increase in the release of hydrolytic enzymes with age (Price, et al., 1971)

Subsequently, with more direct methods for examining single-strand lesions, Massie et al. (1972) hydrodynamically measured the molecular weight of both single- and double-stranded DNA isolated from rat liver cells as a function of age. Molecular weight in both types of molecules decreased approximately ten-fold with increasing age, and this was considered to demonstrate an increase in both single- and double-strand breaks at the molecular level. While this result might appear to be indicative of declining DNA repair processes, it is interesting to note that the largest decrease in molecular weight took place in the first third of the animal's lifespan.

Supporting evidence for increased frequencies of single-strand scissions with age comes from the investigations of Karran and Ormerod (1973). DNA isolated from striated muscles of 28-day old rats had lower molecular weights on alkaline sucrose gradients when compared to striated muscle DNA from one-day old rats. However, as Karran and Ormerod (1973) themselves have suggested, this apparent decrease in molecular weight might have been the result of the preparative procedure and not reflect in vivo conditions.

Wheeler and Lett (1974) applied zonal centrifugation alkaline sucrose gradients to DNA isolated from young and aged dog neurons. With age, the DNA was found to have decreased molecular weights and, again, this result appears to demonstrate an accumulation of unrepaired lesions. More recently, Ono et al. (1976) examined DNA isolated from aging mouse liver, spleen, thymus, and cerebellum by alkaline sucrose gradient centrifugation. Only hepatic DNA was observed to decrease in molecular weight with age. However, this decrease in molecular weight occurred between mice 1-2 and 14 months old. There was no further significant decrease in molecular weight with age.

Finally, the frequency of single-stranded DNA regions in aging mouse liver cells has been measured by Chetsanga et al. (1975) utilizing an assay

based on the single-strand-specific nuclease S_1 . This assay system cannot ascertain the number of such sites present but only the extent to which the total genome is involved. Only after approximately one-half of the measured lifespan (~ 30 months) was an increase in S_1 activity noted which by the end of their lifespan reached almost 25% of the total genome.

3.2. DNA Cross-Linking

The previous investigations, while differing in their assay systems, have been directed exclusively at the presence of denatured regions and/or strand scissions in DNA. Other in vivo investigations, however, have been directed towards examining DNA cross-linking. In one study, pregnant rats were fed tritium and the vital organs (brain and liver) of their offspring were examined at different ages for cross-linked products (Acharya, 1972). With age, not only was there an increase in the frequency of DNA-protein-RNA complexes, but their molecular size also increased. Since some of these substances retained tritium activity, it appeared as if they had remained fixed in the cells from birth and were therefore presumably immune to normal DNA repair processes.

In a similar investigation, Cutler (1976a) observed an accumulation in DNA-protein crosslinks with age in rodent liver tissue and at a rate approximately inversely proportional to lifespan potential in two rodent species-- Mus and Peromyscus.

In support of the formation of DNA crosslinks with age, the percent of DNA which can readily be isolated from aging liver tissue declines (Amici et al., 1974) and DNA isolated from aged organs has a higher melting temperature (von Hahn and Fritz, 1966). Also, Salsar and Balis (1972) examined the extent of DNA-bound protein after extensive extraction procedures in various tissues of aging rats. They observed no change in total bound protein in rat liver,

an increase in spleen and kidney, and a decrease in intestinal tissue as a function of age.

While these in vivo investigations have largely demonstrated an increase in cross-linking with age, one recent in vitro investigation has proved negative. Bradley et al. (1976) examined levels of DNA cross-linking in WI-38 cells, a fetal lung fibroblast cell line with a limited in vitro lifespan, and observed no increase as a function of in vitro passage level. Their assay system, based on filter elution rates of DNA from alkaline lysed cells, is capable of detecting DNA cross-linked not only to complementary strands but also to other cellular constituents (Bradley et al., 1976).

3.3. Chromosome Aberrations

As another indication for an age-associated accumulation of unrepaired DNA lesions, some investigations have been directed towards examining so-called "spontaneous" levels of chromosome aberrations in metaphase cells. Chromosome aberrations can arise in cells from the presence of known unrepaired DNA lesions (Parrington et al., 1971; Sasaki, 1973), and many types of DNA lesions can result in the same general classes of aberrations (Bender et al., 1973a, b, 1974). Consequently, a decline in the efficiency of any DNA repair process should give rise to an increase in spontaneous chromosome aberrations. However, a decrease in DNA repair capability is not the only possible mechanism by which increased spontaneous levels of aberrations could arise. Chromosome aberrations also occur as a result of interference with normal DNA synthesis (Kihlman, 1966). Secondly, this system is, of course, limited to proliferating cellular populations and, of necessity, cannot provide information about the large variety of postmitotic tissues in the aging organism.

In vivo, two major cellular systems have been examined for spontaneous chromosome aberrations as a function of age. Both systems involve the

examination of tissues which are normally quiescent--liver and peripheral lymphocytes--but which can be forced into active mitosis under the appropriate stimulus.

The liver system was initially chosen because it offered the opportunity of examining a tissue which would have the potential of accumulating DNA lesions without any loss due to normal cellular turnover (Curtis, 1963). This system involves the partial destruction of the liver either by subcutaneous injections of carbon tetrachloride (Stevenson and Curtis, 1961) or by partial hepatectomy (Brooks et al., 1973). While the liver is subsequently undergoing regeneration, metaphase or anaphase cells can be examined for chromosome aberrations.

Extensive investigations have observed an age-dependent direct increase in liver chromosomal aberrations in mice (Stevenson and Curtis, 1961), dogs (Curtis et al., 1966), guinea pigs (Curtis and Miller, 1971), and chinese hamsters (Brooks et al., 1973). This age-dependent increase also appears to be inversely proportional to lifespan--increasing at a faster rate in inbred mouse strains with shorter lifespans than in inbred mouse strains with longer lifespans (Crowley and Curtis, 1963). While this proportionality also held true for mice, guinea pigs, and dogs, chinese hamsters appeared to be an exception (Brooks et al., 1973). This discrepancy, however, could be due to differences in ascertainment.

In another in vivo cellular system, peripheral lymphocytes derived from donors of different ages are stimulated with a mitogen, generally phytohemagglutinin (PHA), to divide. After some specific time interval, metaphase cells are then examined for chromosome aberrations. In man, Court-Brown et al. (1966), Tough et al. (1970), Jarvik and Kato (1970), Liniecki et al. (1971), and Ayme et al. (1976) have observed a slight increase in aberration frequencies in the aged populations. On the other hand, Jacobs and Court-

Brown (1966), Sandberg et al. (1967), Bochkov et al. (1968), Goodman et al. (1969) and Bochkov (1972) have all observed no such increase. In swine, a marginal increase in chromosome deletions was observed with increasing age, all other types of aberrations remaining constant in frequency (McFee et al., 1970). However this cellular system is extremely complex in nature and there are significant differences between the replicative rates of stimulated cells in young and aged lymphocyte cultures (Tice, 1976). Consequently, any difference in spontaneous aberration frequencies might be a result of metabolic alterations and not due to increased frequencies in unrepaired DNA lesions.

In proliferating cellular populations in vivo, the data appear to be equally inconsistent. Chlebovsky et al. (1966) reported a dramatic increase in chromosome aberration frequencies in extremely aged rat bone marrow cells. On the other hand, Curtis and Tilley (1971) observed no such increase with age in bone marrow cells derived from either aging mice or rats.

In vitro, such investigations have been largely concerned with examining chromosome aberration frequencies in human fetal-derived fibroblast cell lines as a function of in vitro passage. These cell lines have been observed to undergo increased frequencies of chromosome aberrations, aberrations largely limited to dicentric chromosome formation, during senescence (Sax and Passano, 1961; Saksela and Moorehead, 1963; Thompson and Holliday, 1975; Benn, 1976).^{However} these chromosome aberrations have been suggested to be a consequence of defective proteins and not a major cause of death in senescent cells (Thompson and Holliday, 1975).

All of these investigations, whether directed at single-strand scissions and cross-linking in DNA or aberrations in metaphase chromosomes, have been limited in the number of different types of tissues examined. The only in vivo tissue clearly demonstrated to undergo an age-related increase in

unrepaired DNA lesions has been liver tissue. All investigations into other tissues, in vitro as well as in vivo, have been either entirely negative or not consistently positive. The frequency of cross-linked DNA and chromosome aberrations in the liver appears not to increase abruptly near the end of an animal's lifespan but rather steadily accumulates throughout. This observation would indicate that declining DNA repair processes are not critical for aging but rather that certain proportions or types of DNA lesions escape repair and that these lesions accumulate within a cell, possibly doing irreparable damage to its normal function. This increase in unrepaired lesions might, however, be tissue-specific and not reflect general in vivo conditions during aging.

4. DNA REPAIR CAPABILITY AS A FUNCTION OF AGE

In an attempt to correlate aging with a possible decline in DNA repair capability, numerous investigators have examined the efficiency of various repair systems in aging cellular systems in vitro as well as in vivo.

In vitro, investigations have been primarily concerned with assessing DNA repair levels in human- and animal-derived fibroblast cell lines as a function of the number of population doublings passed. Human cellular research has been largely directed at comparing DNA repair capacity in cultured lymphocytes, or in a few instances cultured skin fibroblasts, derived from donors at different ages. Animal research in vivo has focused on a few post-mitotic tissues as well as some proliferating cellular systems.

4.1. In vitro Investigations

4.1.1. Human Cellular Systems

Goldstein (1971b) was the first to investigate the question of DNA repair capability as a function of in vitro passage level. Using UV-induced UDS and

cell survival as measurements of DNA repair competence, he observed no decline in repair capability in human skin fibroblast cell lines until very near the end of their in vitro lifespan. This finding was soon confirmed in the human embryonic lung fibroblast cell line (WI-38) by Painter et al. (1973). Subsequent investigators have observed some decrease in UDS measured excision repair with increasing in vitro passage levels of WI-38 cell populations (Hart and Setlow, 1976; Bowman et al., 1976). Both Hart and Setlow (1976) and Bowman et al. (1976) concluded that the observed decline correlated with the number of cells no longer capable of undergoing normal semiconservative DNA replication. While this conclusion suggests some sort of direct relationship between the two processes, it is impossible to determine which process is a consequence of the other.

Single-strand break rejoining has also been extensively examined in human fibroblast cultures at different in vitro passage levels. Clarkson and Painter (1974) examined x-ray-induced single-strand break rejoining in WI-38 cells with both alkaline sucrose gradient centrifugation and UDS techniques. They observed no repair decline throughout the in vitro lifespan. In agreement with Clarkson and Painter (1974), Bradley et al. (1976) observed normal rates of single-strand break rejoining in terminally senescent WI-38 cultures as assayed by both alkaline elution and alkaline sedimentation techniques.

In human skin fibroblasts, Epstein et al. (1973b, 1974) and Little et al. ^{strand} (1975) observed a decline in the rate of/rejoining by alkaline sucrose gradient centrifugation as the cells approached senescence. While this decline in DNA repair capacity was more marked in cells at the end of their in vitro lifespan, it appeared to commence prior to any indication of terminal senescence (little, 1976).

Finally, in one recent study, Mattern and Cerutti (1975) examined aging WI-38 cell nuclei and nuclear sonicates for their ability to specifically excise osmium tetroxide- or gamma-induced 5,6-dihydroxydihydrothymine residues. Late passage cells exhibited a complete loss in the ability to excise this specific type of DNA lesion and this loss in repair occurred prior to complete cessation in growth.

4.1.2. Animal Cellular Systems

Examination of animal cellular systems for DNA repair capability during their in vitro lifespan has been largely limited to fibroblast cell lines of fetal origin. In mouse embryo fibroblast cultures, UV-induced UDS and pyrimidine dimer release declined only in terminally senescent cultures (Ben-Ishai and Peleg, 1974). Transformed cells from these senescent cultures did not regain the loss in DNA repair capability.

Paterson et al. (1974) examined UV-induced UDS in a chick fibroblast system and observed a lack of excision repair in middle- or late-passage cells when compared to early passage populations.

Finally, Williams and Little (1975) have investigated both UV- and gamma-ray-induced UDS as well as single-strand break rejoining by alkaline sedimentation in primary hamster embryo cultures. Active UV- and gamma-induced UDS was present throughout the in vitro lifespan of these cellular populations, ceasing only in terminally senescent cultures. Single-strand break rejoining rates, however, appeared to decline between early and late passage populations. In this cell system, transformed cells retained both the UDS activity and single-strand break rejoining rates of early passage cells.

The results of these investigations on DNA repair capability during the in vitro lifespans of explants derived from both human and animal tissues

are somewhat conflicting. The possibility exists, however unlikely, that these differences are simply a consequence of increased resolution in some of the assay systems used to examine DNA repair. However, the majority of the investigators observed no appreciable decline in either excision repair or single-strand break rejoining until after senescence had begun. Furthermore, through double-labeling autoradiography the repair decline that was observed in senescent cellular populations correlated strongly with a simultaneous loss in cells for their ability to undergo normal DNA synthesis. This finding suggests that the observed in vitro DNA repair decline may be a consequence of the metabolic changes in senescent cells and not the direct cause of in vitro senescence per se.

4.2. In Vivo Investigations

4.2.1. Human Cellular Systems

Research connected with examining DNA repair capability in humans as a function of age has been limited to two cellular systems: skin fibroblast cell lines and mitogen-stimulated peripheral lymphocyte cultures. Due to their greater accessibility, cultured lymphocytes have been more often examined for alterations in DNA repair capability. Liniecki et al. (1971) examined G_1 x-ray induced chromosome aberration yields in PHA-stimulated lymphocytes from donors ranging in age from zero to seventy-two years. No differences were observed in the yields of acentric fragments, rings, and intercalary deletions between young and aged donors. Only dicentric frequencies appeared to change, but in a negative manner, as a function of age.

Chromosome aberration induction by exposure to chemical agents has also been examined in lymphocyte cultures from young and aged individuals. Bochkov and Kuleshov (1971, 1972) exposed stimulated lymphocytes to

thio-tepa and degranol and observed that the frequency of induced aberrations increased significantly in the aged population. In a similar finding, Lambert et al. (1976) measured UV-induced UDS levels and found a 25% decline in repair synthesis in lymphocytes from individuals older than sixty years. However, not only do stimulated lymphocytes from young and aged donors cycle at different rates (Tice, 1976) and chromosome aberration yields vary in metaphase cells depending upon their replicative history (Tice and Ishii, 1977), but levels of UV-induced excision repair in this system depend upon the degree and extent of lymphocyte stimulation (Darzynkiewicz, 1971). Consequently, these results might be artifactual in nature and not reflect the true state of DNA repair capacity.

Only Goldstein (1971) has published any data in which DNA repair levels are compared in skin-fibroblast cultures derived from young and aged donors. Goldstein (1971) examined cell survival after UV-exposure in early passaged cultures of fetal, newborn, young, and aged origin and observed no significant differences in UV-sensitivity.

4.2.2. Animal Cellular Systems

Examining gamma-ray induced chromosome aberrations in swine lymphocyte cultures from young and aged animals, McFee et al. (1970) observed no increase in chromosome-type aberrations (i.e. achromatic lesions, fragments, rings, or dicentrics) but did observe an increase in chromatid-type aberrations (i.e. breaks or achromatic lesions).

In order to assess DNA repair in an actively proliferating cellular population in vivo, Curtis and Tilley (1971) irradiated mice and examined chromosome aberration frequencies in metaphase cells from the bone marrow. Not only was there no difference in induced aberration yields between young and aged mice, but the aberration frequencies declined with time at the same rates.

Finally, two post-mitotic tissues have been examined for a possible decline in DNA repair capability. Wheeler and Lett (1974) assessed the capability of internal granular-layer neurons from beagles of different ages to repair gamma-ray induced single-strand breaks by alkaline sucrose gradient sedimentation. No age-associated decline in this type of repair ability was observed. Yet, when isolated myocardial cells isolated from newborn and adult rats were compared for UV-induced UDS levels, the adult cells exhibited a complete loss in capability (Lampidis and Schaiberger, 1975). However, since the newborn rat myocardial cells still evidenced some normal DNA synthesis, this finding could be attributed to the normal process of differentiation and not to aging. (See following section.)

These results derived from both human and animal models suggest that there is no appreciable age-related decline in, at least, the ability to repair damage induced by ionizing radiation in either post-mitotic, proliferating, or quiescent cellular populations. There is some suggestion that in stimulated lymphocytes from aged humans there is increased sensitivity to certain chemical compounds. However, with these chemical agents, it is difficult to interpret the actual findings. The apparent differences in chromosome aberration yields could be the result of differences in mutagen metabolism, or due to differences in the replication kinetics of the cultures. Investigations examining excision repair are too few and too conflicting to be helpful. There is no doubt that more investigations are necessary in this area before any reliable conclusion can be made.

5. TISSUE-SPECIFIC DNA REPAIR CAPABILITY

In any discussion of a possible correlation between aging and DNA repair capability, some consideration must be given to the possible existence of tissue-specific differences in repair levels within the organism.

Several investigations have suggested that non-proliferating, terminally differentiated cells undergo a loss in DNA repair capacity when compared with proliferating precursor cells. Rat- or embryonic chicken-derived skeletal muscle cells suffer a significant loss in UDS measured repair replication as they fuse in vitro into multinucleated cells and myotubes (Hahn et al., 1971; Stockdale, 1971; Stockdale and O'Neill, 1972). In a more extensive investigation, Chan et al. (1976) examined both single-strand break rejoining and UDS measured-repair replication in an established myoblast cell line before and after in vitro induced fusion. Both the rate and overall levels of 4-nitroquinoline (UV-like)-induced UDS were reduced by more than 50% after myotube formation. This loss was due in part to a complete cessation in the ability of myotube cells to recognize and repair alkali-sensitive lesions. On the other hand, myotubes showed no decline in their ability to repair x-ray induced single-strand breaks. Also, developing chick retinoblasts undergo a loss in UV-induced UDS capacity (Strauss, 1976) and mouse neuroblastoma cells become increasingly sensitive to UV exposure during differentiation (McCombe et al., 1976).

In at least one system, this loss in DNA excision repair capability with differentiation has been demonstrated to be recoverable. The mouse neuroblastoma cell system of McCombe et al. (1976) is capable of undergoing reversible differentiation in vitro depending upon serum concentration. These cells regain normal levels of UV-resistance after dedifferentiation but not until after the first mitosis has been completed.

This correlation with proliferative activity and excision repair competence also occurs with peripheral lymphocytes. These cells exhibit dramatic increases in their ability to remove acetoxy-aminofluorene-induced damage (Strauss, 1976; Scudiero et al., 1976) and undergo UV-induced UDS

(Darzynkiewicz, 1971) after mitogen stimulation. Although not equal in magnitude, this increase in repair competence occurs in parallel with increasing levels of DNA polymerase activity (Scudiero et al., 1976).

This loss in DNA repair replication capacity with differentiation expresses itself in comparative tissue studies. Examinations of UV-induced UDS revealed that peripheral lymphocytes have a greater capacity than peripheral granulocytes (Darzynkiewicz, 1971) and fibroblasts have a greater capacity than pulsating cardiac cells from the same rat organ (Lampides and Little, 1976). On the other hand, cultured bovine lens epithelial cells and lung fibroblast cells from the same animal have equal capacities for repairing x-ray induced single-strand scissions (Treton and Courtois, 1976).

These various investigations into DNA repair capability as a function of the proliferative state of a cellular population support the conclusion that post-mitotic cells suffer a loss in excision repair but not in DNA strand break rejoining. Whether or not some positive correlation exists between DNA polymerase activity and DNA excision repair capability, different tissues vary qualitatively and quantitatively in their inherent abilities to repair specific DNA lesions. These differences correlate strongly with proliferative activity. This loss in excision repair capacity concomitant with a loss in proliferation during differentiation suggests that perhaps some of the apparent decline in DNA capability observed in some proliferating cellular systems as they approach senescence (e.g. WI-38) could be directly due to a loss in proliferative activity. If DNA repair capability is critical to the aging process then some variation in the aging rate might be expected to occur between different tissues since not all tissues are equally repair competent. Because differentiated tissues exhibit the lowest repair capability, tissues such as neuronal or muscle should age fastest. In fact,

in vivo aging does appear to be more conspicuous in certain tissues, particularly those involving differentiated post-mitotic cells (Franks, 1974; Aune, 1976).

6. ACCELERATION OF THE AGING PROCESS BY EXPOSURE TO DNA DAMAGING AGENTS

The acceleration of the aging process by ionizing radiation has long been held as fundamental to the relationship between DNA repair, mutational events and aging (Yielding, 1974). If aging is the consequence of an accumulation of unrepaired DNA lesions, then exposure of animals to agents capable of damaging DNA should accelerate the aging process. Early evidence did suggest that radiation-induced life-shortening was mechanistically related to normal aging. Exposure of animals to ionizing radiation appeared to prematurely age their physical appearance, advance the incidence of age-related biochemical changes, and increase the mortality rate by accelerating the time of onset for many diseases (Alexander, 1957; Upton, 1957; Lindop and Rotblat, 1961a, b; Casseratt, 1964).

However, extensive reassessments of all phases of radiation-induced life-shortening have suggested that this phenomenon is substantially different from the normal aging process (Alexander, 1967; Walburg, 1975). While high radiation doses accelerate the appearance of non neoplastic diseases such as nephrosclerosis, anemia, and sterility (Upton et al., 1960), no evidence has been found for such effects after single, acute exposures below 300 R when corrections for competing probabilities of death are made (Walburg, 1975). Yet, these exposure levels cause significant life-shortening in the irradiated populations (Walburg, 1975). Exposure of animals to ionizing radiation appears to increase the incidence of cancer in most organs, resulting in premature death. When survivor data are analyzed with the deaths from neoplasia removed, there are no differences between the life

expectancy of the treated and of the controls (Hoel and Walburg, 1972; Walburg and Hoel, 1972).

In addition, there does not appear to be any proportional advancement in time of many of the morphological and physiological manifestations of the normal aging process in animals (Berch and Curtis, 1964) or in man (Conard et al., 1966; Hollingworth/et al., 1969). Classical senescent events such as alterations in collagen, pigment accumulation, and neuromuscular function appear to be refractory to irradiation (Walburg, 1975).

Similar to radiation experiments, the possible effects of chemical mutagenic agents on life-shortening and aging have been examined. Alexander and Connell (1960) observed that chlorambucil, a nitrogen mustard compound, and Myleran, an alkylating agent, shortened the lifespan of mice but did not hasten the onset of any typical aging manifestations. Conklin et al. (1963) concluded that mid-lethal doses of nitrogen mustard shortened lifespan, and although it was not the result of increases in specific neoplasias, the process was still not equivalent to normal aging. Curtis (1963) and Stevenson and Curtis (1961) subjected mice to either a generalized or an acute stress with nitrogen mustard or typhoid toxoid. In both instances, the life expectancy of the mice was not significantly altered. Finally, Alexander (1967) exposed mice to ethylmethanesulphonate, a known mutagen, without any evidence for life-shortening. The conclusion appears to be that, while some mutagenic compounds can cause life-shortening, there exists no evidence for accelerated aging.

Radiation does, however, appear to accelerate aging at the cellular level in in vitro systems. Lima/et al. (1972) examined the long term effects of Cobalt-60 on the growth potential of chick embryo fibroblastic lines, cell lines which normally have a limited potential for divisions in vitro and

which attain lower cellular densities at confluence as a function of age. The effect of radiation exposure was both irreversible and cumulative--shortening the in vitro lifespan while also decreasing saturation densities. et al. Maciera-Coelho/(1976) has observed that exposure to ionizing radiation shortens the in vitro lifespan while concurrently accelerating the acquisition of an infinite growth potential (transformation) in those cell lines capable of such a process. It would appear that exposure to ionizing radiation has accelerated at least two normal in vitro aging parameters. However, the possible relationship between these in vitro observations and in vivo studies have not been explored.

It would appear that the life-shortening induced by ionizing radiation or mutagenic compounds differs both quantitatively and qualitatively from the normal aging process. The life-shortening observed after exposure of animals to agents capable of inducing DNA damage is generally a consequence of increased frequencies of neoplasia. The conclusion that many of the normal aging parameters are not present during this life-shortening process suggests that the accumulation of mutational events is not fundamental to the aging process. However, it does suggest that certain of the age-related components, such as the observed increase in neoplasia, might be a consequence of the presence of unrepaired DNA lesions. Perhaps this is an indication that different tissues in an organism not only age at different rates but age for different reasons.

7. HUMAN GENETIC SYNDROMES

If DNA repair processes are in fact basic to the aging process, then it could be expected that genetic syndromes with known defects in specific types of DNA repair would also express some characteristics of accelerated aging. These characteristics could be expressed in vivo as premature aging

and in vitro as an early loss in replicative ability in fibroblast cultures. Three genetic syndromes are known to suffer a loss in DNA repair capability-- ataxia telangiectasia, Fanconi's anemia, and xeroderma pigmentosum. These and possible related evidence for accelerated aging will be discussed in the following pages.

In addition, two genetic syndromes which appear to predispose the individual toward premature aging will be examined for evidence of decreased DNA repair capability: progeria and Werner's syndrome.

7.1. DNA Repair Deficiency Syndromes

7.1.1. Ataxia Telangiectasia

Ataxia telangiectasia (or the Louis-Barr syndrome) (AT) is an autosomal recessive trait whose general clinical manifestations include a progressive cerebellar ataxia (loss of muscular control), oculocutaneous telangiectasia (chronic dilation of small blood vessels), recurrent sinopulmonary infections, a consistent defect in cellular immunity, thymic abnormalities, and increased disposition toward neoplasia, particularly of the reticuloendothelial system (Tadjoedin and Fraser, 1965; Kersey et al., 1973; Peterson et al., 1966; Bochkov et al., 1974).

This syndrome exhibits a slight but significant increase in spontaneous chromosome aberration frequencies in both cultured lymphocytes and fibroblast cultures (Hecht et al., 1966; Gropp and Flatz, 1967; Schroeder and Kurth, 1971; Bochkov et al., 1974; Harnden, 1974; Oxford, 1975; Cohen et al., 1975). However, some investigators have observed no significant increase in spontaneous aberrations in cultured lymphocytes from AT patients (Pfeiffer, 1970; Schmid and Jerusalem, 1972) suggesting possible genetic heterogeneity. Other investigators have reported increased chromosome

aberration frequencies in cultured lymphocytes but not in direct bone marrow preparations (Hecht et al., 1973) or skin fibroblasts (M. A. Bender, personal communication) from the same affected individual.

AT lymphocytes have been reported to display increased aberration yields after exposure to ionizing radiation in both the G_0 (or G_1) (Higurashi and Conen et al., 1973) parts of the cell cycle. This increase in chromosome aberration frequencies after ionizing radiation has its counterpart in decreased cell survival curves in vitro after gamma irradiation (Taylor et al., 1975) or exposure to radiomimetic chemicals (Hoar and Sargent, 1976) and also in increased radiosensitivity in vivo (Oxford et al., 1975).

The basic repair defect does not appear to involve rejoining of x-ray-induced single-strand breaks (Taylor et al., 1975; Vincent et al., 1975; Paterson et al., 1976) or UV-induced excision repair (Paterson et al., 1976) but rather recognition and repair of gamma-ray induced base damage (Paterson et al., 1976; Taylor et al., 1976).

There exists no definitive evidence for the presence of accelerated aging in individuals affected with ataxia telangiectasia. In vivo, only one report provides some indication of premature aging in individuals affected with this syndrome. Reye and Mosman (1960) observed an advanced loss in immediate memory in one patient which was interpreted to suggest premature senility. No published evidence suggesting accelerated aging in vitro exists to this author's knowledge.

7.1.2. Fanconi's Anemia

Extensive investigations into the genetics of Fanconi's anemia (FA) have shown that this syndrome is inherited as an autosomal recessive trait with some suggestion of genetic heterogeneity (Schroeder, 1966; Remsen and Cerutti, 1976; Schroeder et al., 1976). The syndrome manifests itself as a

progressive, fatal panmyelopathy associated with multiple congenital anomalies, impaired body growth, and kidney malformations (Schroeder et al., 1976).

A characteristic feature of FA, as with AT, is an increased frequency of spontaneous chromosome aberrations (Schroeder et al., 1964). These chromosomal changes have been observed in cultured lymphocytes, in skin fibroblasts and in direct bone marrow preparations (Swift and Hirschhorn, 1966; Schmid, 1967; Sasaki and Tonomura, 1973). Chromosome aberrations appear to consist predominantly of chromatid and chromosome breaks with a relatively small number of exchange type abnormalities (German, 1972).

In an attempt to correlate this increase in spontaneous chromosome aberrations with a possible defect in DNA repair processes, Schuler et al. (1969) demonstrated an increased susceptibility to aberration induction after exposure of FA cells to the alkylating agent tetramethanesulfonyl-d-manitol. This initial observation was confirmed and extended by Sasaki and Tonomura (1973) who found that FA cells were abnormally sensitive to DNA cross-linking agents (e.g. mitomycin C or nitrogen mustard) but not to monofunctional reacting agents (e.g. methylmethanesulphonate). Recently, Latt et al. (1976) had demonstrated a reduced ability in cultured lymphocytes from affected individuals to complete sister chromatid exchanges after exposure to mitomycin C, suggesting a deficiency in replication repair.

There have also been some published reports suggesting an increased sensitivity of both G_0 (or G_1) lymphocytes and fibroblasts from affected individuals to ionizing radiation (Higurashi and Conen, 1971, 1973). However, this observation was not confirmed by Sasaki and Tonomura (1973). Recently, FA cultured lymphocytes were demonstrated to exhibit significantly increased

aberration yields over control cells after G₂ irradiation (M. A. Bender, personal communication) and two of four FA fibroblast cell lines were found to be deficient in selectively excising gamma-induced products of the 5,6-dihydroxydihydrothymine type (Remsen and Cerutti, 1976). Similarly, investigations into UV sensitivity have been equally conflicting. Sasaki and Tonomura (1973) observed no increased UV sensitivity in cultured lymphocytes, yet a fibroblast cell line derived from an affected individual has been characterized as being inefficient in excising UV-induced pyrimidine cyclobutane dimers (Poon et al., 1975).

As with ataxia telangiectasia, there are to this author's knowledge no published reports on FA that suggest any evidence for accelerated aging in vivo or in vitro. While fibroblast cell lines from affected individuals have been reported to have greater population doubling times than control cells (Elmore and Swift, 1976), Vincent and Huang (1976) have observed no curtailment of in vitro lifespan.

7.1.3. Xeroderma Pigmentosum

Xeroderma pigmentosum (XP) is actually a generic term for a family of human skin diseases inherited in an autosomal recessive fashion. They are all characterized by a marked disposition to skin carcinomas and melanomas after exposure to sunlight. Associated with the skin disorders are a spectrum of neurological abnormalities ranging from mild areflexia to severe microcephaly with progressive mental deterioration (the deSanctis-Cacchione syndrome) (Cleaver, 1974; Robbins et al., 1974; Cleaver and Bootsma, 1975; Cleaver et al., 1975).

Both fibroblasts and lymphocytes from affected individuals were demonstrated to display little or no UV-induced UDS (Cleaver, 1969, 1970; Burk et al., 1969). Subsequent investigations have determined the basic

molecular defect to be in the recognition and excision of UV-induced pyrimidine cyclobutane dimers (Setlow et al., 1969; Cleaver, 1969; Cleaver and Bootsma, 1975). Recently, Sutherland (1975) has also shown XP cell lines to be deficient in photoreactivating enzyme levels. XP cells also exhibit reduced UDS repair levels after exposure to various carcinogenic N-oxides (Stich et al., 1973). These cells are, however, competent in rejoining single-strand breaks induced by ionizing radiation (Cleaver, 1969; Kleijer et al., 1973). Further extensive studies utilizing cell hybridization techniques have resulted in the detection of five complementation groups, accounting for the observed heterogeneous clinical manifestations (Kraemer et al., 1975; Cleaver and Bootsma, 1975). Recently, a class of individuals have been reported who exhibit some of the clinical manifestations of XP but whose cells show normal levels of UV-induced UDS (Cleaver and Bootsma, 1975). Lehman et al. (1975) determined that a DNA repair defect existed in the post-replication repair of UV-induced damage.

In contrast to FA or AT, spontaneous chromosome aberration frequencies do not appear to be elevated in cells from individuals with XP. Read et al. (1969), Cleaver and Bootsma (1975), and Parrington et al. (1976) observed no increase in spontaneous aberration frequencies in fibroblast cell lines or cultured lymphocytes from affected individuals. However, Mikhelson (1976) has reported elevated frequencies and Huang et al. (1975) found significantly increased levels of spontaneous aberrations in XP fibroblast cell lines toward the end of their in vitro lifespan.

Cleaver and Bootsma (1975), in their review of XP, concluded that neither XP patients nor XP cells in vitro exhibit any of the expected signs of premature aging. In vitro, XP cell lines show no evidence for fewer total population doublings than age-matched control cells (Goldstein, 1971;

Vincent and Huang, 1976). In vivo, however, while affected individuals do not possess all the characteristics normally associated with aging, they do exhibit some clinical manifestations.

All XP individuals develop pigmentation abnormalities and signs of aged skin, including cutaneous malignancies (Robbins et al., 1974; Cleaver and Bootsma, 1975). This would be consistent with the interpretation that the changes in the appearance of the skin associated with the aging process are largely produced by exposure to UV radiation in sunlight (Kligman, 1969). Furthermore, individuals affected with the more severe neurological variants exhibit progressive abnormalities of internal organs comparable to those observed in the aged (Robbins et al., 1974). These clinical manifestations appear to correlate well with the severity in loss of excision repair capability (Andrews et al., 1976; Takebe, 1976), suggesting a causal relationship between the two processes.

7.2. Accelerated Aging Syndromes

7.2.1. Progeria

Progeria, or the Hutchinson-Gilford syndrome, is a relatively rare autosomally recessive condition that displays a number of clinical symptoms suggestive of accelerated aging. Mean lifespan is dramatically shortened and death is generally a consequence of severe atherosclerosis with cardiac and cerebral involvement (DeBusk, 1972). While thinning of the skin, atherosclerosis, and the loss of subcutaneous fat (DeBusk, 1972) are representative of accelerated aging, other features are not. These features include delayed dentition, presbyopia, articular cartilage destruction (DeBusk, 1972), and a lack of elevated tissue lipofuscin levels (Spence and Herman, 1973).

In vitro, progeric skin fibroblast cultures have been reported to undergo significantly fewer population doublings than control cells (Singal and Moerman and Goldstein, 1973; Goldstein/ 1976). However, other investigators have observed relatively normal in vitro lifespans (Martin et al., 1970; Danes, and Moerman 1971). Goldstein/(1976) have observed significant increases in altered enzyme levels in progeric fibroblast cells, levels in excess of those normally observed in senescent fibroblasts in vitro (Holliday and Tarrant, 1972). However, there does not appear to be any corresponding increase in altered morphology (Danes, 1971) that is normally observed in aging fibroblast cultures (Robbins et al., 1970; Lipetz and Cristofalo, 1972).

Although these observations suggest that there are quantitative and qualitative differences between normal aging and the presumed accelerated aging observed in progeria, several investigators have used progeric cell lines to assess possible aging parameters (Epstein et al., 1973a Goldstein/ and Moerman 1976). Epstein et al. (1973a) proposed that the basic defect in progeria could be the result of a deficient DNA repair capability. These investigators

observed a marked decrease in the ability of progeric fibroblast cells to repair x-ray-induced single-strand breaks. / However, this initial finding was not confirmed by Regan and Setlow (1974) examining the same cell strain.

Subsequently, Epstein et al. (1974) examined six progeric cell lines for single-strand break rejoining and found two lines to be nearly normal and two cell lines to be markedly deficient in repair capacity. Bender and Rary (1974) observed no increase in chromosome aberration yields of x-irradiated cultured lymphocytes from the progeric patient examined by Epstein et al. (1973). Most recently, Bradley et al. (1976) observed no defective rejoining of single-strand breaks assayed through alkaline elution and alkaline sedimentation technique, in a progeric fibroblast cell line.

The evidence suggests that progeria does not suffer from an intrinsic defect in DNA repair but rather a loss in capability as a consequence of abnormal metabolism and/or some type of genetic repression. This would be consistent with the finding that SV₄₀ transformation of progeric cells restores normal ability in rejoining of single-strand breaks (Little et al., 1975).

7.2.2. Werner's Syndrome

This syndrome, inherited as an autosomal recessive trait, has its onset in individuals between the ages of fifteen and twenty years (Epstein et al., 1966; Zucker-Franklin et al., 1968). Some of the clinical features of this syndrome/resemble premature or accelerated aging /marked lymphoid depletion, glucose intolerance, cataracts, graying of the hair, increased incidence of neoplasms and arteriosclerosis (Epstein et al., 1966; Zucker-Franklin et al., 1968). However, as in progeria, clinical features exist which are qualitatively and quantitatively different from those observed in the elderly. Finally, affected individuals also suffer a delayed sexual maturity (Epstein et al., 1966).

In vitro, fibroblast cell cultures routinely exhibit poor growth, decreased lifespan, and accelerated morphological alterations similar to those observed in senescent normal fibroblast cultures (Stecker and Gardner, 1970; Epstein et al., 1966; Martin et al., 1970; Danes, 1971). Increased levels of altered enzymes have also been reported for these fibroblast cell lines, consistent with the notion of premature aging (Holliday et al., 1974; Goldstein and Singal, 1974; Goldstein /1976) and Moerman

There exists no published evidence of a possible defect in DNA repair capability associated with this syndrome. While this cannot be considered proof that one does not exist, it is suggestive.

Some tentative conclusions can be drawn from the nature of these inherited human syndromes. First, in those syndromes which appear to lack some aspect of normal DNA repair capability, only xeroderma pigmentosum exhibits any evidence of premature or accelerated aging. However, this accelerated aging is not of a general nature but appears to be specifically limited to two tissues--skin and neural. Despite their increased frequencies of spontaneous chromosome aberrations, neither ataxia telangiectasia nor Fanconi's anemia provides good evidence for any features resembling premature aging. These observations suggest either a lack of correlation between the two processes or possible differences in importance of specific DNA repair systems to aging.

Secondly, while neither progeria nor Werner's syndrome can be equated in a simple fashion to premature aging in vivo, they do have some aspects in common with normal aging. Consequently, the lack of any definitive evidence demonstrating the presence of DNA repair deficiencies suggests the two processes might be intrinsically unrelated.

8. LONGEVITY AND DNA REPAIR PROCESSES

It has been previously noted that different species exhibited differences in their ability to excise UV-induced thymidine dimers. Human- and bovine-derived cells were extremely competent in excision repair (Cleaver et al., 1972; Regan et al., 1971) when compared to rodent-derived cells (Painter and Cleaver, 1969; Rauth, 1970). These differences led Hart and Setlow (1974) to investigate a possible correlation between extent of excision repair in an adult-derived tissue and mean lifespan. It was expected that differences in longevity may be a manifestation of more efficient repair processes in those species with greater lifespans. Since the magnitude of excision repair depends on the state of the particular cell examined (see section 5), these investigators examined only primary fibroblast cell lines at early in vitro passages. Analysis of UV-induced UDS levels in a number of different animal species resulted in a strong correlation between species' lifespan and levels of excision repair (Hart and Setlow, 1974; Hart, 1976).

However, when this correlation was further tested within primates, a closely related group of species in which total lifespan varies by more than twenty-fold (Cutler, 1975b), no differences in UV-induced UDS levels were observed (H. Kato, personal communication). Consequently, the apparently strong correlation between longevity and excision repair might be nothing more than an experimental artifact. Alternatively, the data might suggest that while differences in mean lifespan do tend to reflect intrinsic levels of excision repair, other processes can modify the aging rate.

These studies have exclusively investigated excision repair capability. However, an accurate assessment of repair capability requires the examination of all DNA repair systems present. Although rodent-derived cell lines are considered to be excision repair deficient they are obviously not DNA repair incompetent since they do not appear to suffer from

a lack of this repair process. Also, it is difficult to firmly assess the correlation between excision repair and lifespan from investigations restricted to one tissue, skin fibroblasts. The possibility exists that other tissues will not express the same strong correlation between DNA repair and mean lifespan.

SUMMARY AND CONCLUSION

The existence of a causal relationship between DNA repair capability and aging is based on the assumption that aging is, in fact, a direct consequence of the detrimental accumulation of unrepaired DNA lesions at the cellular level. Presumably, when the frequency of these lesions reaches a certain level and/or specific genes become inactive, loss in cellular function ensues. As the proportion of such cells increases in various tissues, the organism "ages". If this assumption is a valid one, then the more effective a cell is in preventing and/or repairing DNA damage, the slower the aging process should be.

It is possible to envision two separate, but not/^{mutually}exclusive mechanisms, by which unrepaired DNA lesions could accumulate with time. First, a certain proportion of DNA lesions might escape detection and/or repair at any one time. Assuming that both repair capabilities and environmental conditions do not vary significantly with time, unrepaired DNA lesions should then tend to accumulate in a cell at a rather constant rate. The second mechanism also assumes no significant alteration in the number of potential lesions induced, but rather that the proportion of successful repair events diminishes with time as a consequence of declining DNA repair capability. In this event, unrepaired DNA lesions should increase at substantially faster rates as the level of repair capability becomes more inefficient with advancing age. Both mechanisms would give rise to levels of DNA lesions

capable of incapacitating a particular cell. However, the former mechanism suggests that lifespan is related to the intrinsic capacity of the cells in an organism to repair DNA damage and that different organisms with different repair capabilities will have different lifespans. The latter mechanism, while not negating the former, suggests that lifespans could be longer except for the decline in DNA repair capability.

Primarily, investigations concerned with determining frequencies of unrepaired DNA lesions have only compared tissues derived from animals at the two extremes of the aging process. A further difficulty arises from the rather limited distribution of tissues examined. However, some conclusions concerning both the occurrence and the rate of accumulation of unrepaired DNA lesions in different tissues can be made.

First, not all tissues have been found to exhibit an age-related increase in DNA lesions. DNA strand breakage and/or alkaline-labile regions do not appear to increase with age in mouse spleen, thymus, or cerebellum (Ono et al., 1976). Spontaneous chromosome aberrations exhibit no consistent increase in frequency in proliferating bone marrow cells with age in rats or mice (Curtis and Tilley, 1971) or in lymphocytes cultured from human donors of different ages (Sandberg et al., 1967; Goodman et al., 1969; Bochkov et al., 1968, for example). In vitro studies have proved equally negative both with respect to an increase in DNA cross-linking (Bradley et al., 1976) or with significant increases of spontaneous chromosome aberrations (Thompson and Holliday, 1975) as a function of passage level.

Secondly, where unrepaired DNA lesions do exhibit increased frequencies in an aging cellular system (e.g., liver), they tend to accumulate throughout the animal's lifespan and not dramatically near the end of it. The increases in strand breakage observed in mouse liver cells are more significant in the early

portions of the ^{animal's} lifespan than in the later ones (Massie et al., 1972; Ono et al., 1976). The increase in both DNA cross-linking and spontaneous chromosome aberrations in liver cells appears to be both roughly linear with age and inversely proportional to the organism's lifespan (Cutler, 1976; Stevenson and Curtis, 1971; Curtis et al., 1966; Curtis and Miller, 1971).

These observations suggest a ^{lack of a} decline of DNA repair capability with age, and, broadly speaking, investigations concerned with directly assessing DNA repair efficiency are in accord. In vivo, no significant increase in x-ray-induced chromosome aberration yields was observed in lymphocytes cultured from young to aged individuals (Liniecki et al., 1971). Similarly, neurons from aged and young beagles proved equally capable of rejoining gamma-ray-induced single strand breaks (Wheeler and Lett, 1974) and skin fibroblast from aged donors exhibited no increased sensitivity to UV light as measured by cell killing (Goldstein, 1971). However, a decline in UV-induced UDS has been reported by Lambert et al. (1976) and an increase in chemically-induced chromosome aberrations (Bochkov and Kuleshov, 1971, 1972) in lymphocytes from aged donors. In vitro investigations concerned with the efficiency of DNA strand break rejoining have resulted in conflicting conclusions. Some investigations have observed no decline in this type of repair ability until in vitro senescence had already begun (Clarkson and Painter, 1974; Bradley et al., 1976) while others have reported the presence of a decline prior to senescence (Epstein et al., 1973b, 1974; Little et al., 1975; Williams and Little, 1975). Excision repair, however, appears to decline only after the onset of in vitro senescence and appears to correlate with a loss in replicative ability (Hart and Setlow, 1975; Williams and Little, 1975; Bowman et al., 1976).

The accumulated data, while not as extensive or as consistent as one would like, does not firmly support any age-related decline in DNA repair capability. A large variety of cellular systems exhibit either no increase in unrepaired DNA lesions or no loss in DNA repair capability with age.

The only cellular system to consistently exhibit an increase in DNA lesions with age is liver tissue in vivo. DNA strand breakage, DNA cross-linking, and spontaneous chromosome aberrations all increase in frequency in liver tissue beginning early in the animal's lifespan. However, the importance of the conclusion that the increase of DNA lesions in liver cells is inversely proportional to the animal's lifespan must be examined in terms of a general aging process. It is extremely difficult to demonstrate an age-related loss in liver function (Thompson and Williams, 1965; Thung and Hollander, 1967) and liver failure is generally not a cause of death in aging animal populations (Kohn, 1966). The conclusion that lifespan does not depend upon either the number or percent of altered liver cells suggests that the general accumulation of DNA lesions might rather be a consequence of liver specific metabolic processes. It is interesting to note that a strong correlation has been found to exist between lifespan and the ability of cultured fibroblast to metabolize 7,12-dimethylbenz(A) anthracene to its mutagenic metabolites (Schwartz, 1975). Consequently, the rate of DNA lesion accumulation in liver cells might merely reflect enzyme levels and not lifespan specific repair capabilities.

The lack of correlation between aging of the liver and age-related mortality suggests that other tissue might be of greater importance to the aging process. Aging does not appear to be a consequence of a decline in tissues that normally proliferate (e.g., bone marrow) or in tissues that retain the capacity to proliferate in response to a specific stress

situation (e.g., liver) (Kohn, 1973; Franks, 1974; Cutler, 1976). Aging appears to be more conspicuous in terminally differentiated postmitotic tissues such as neural or muscle tissue (Aune, 1976). In fact, a significant proportion of natural deaths can be attributed to degenerative age-associated alterations in the central nervous system (Burnett, 1974). Aging of these tissues does not appear to be the result of an actual loss in cell number (Kohn, 1973; Cameron and Thrasher, 1976) but rather to a continuous decline in functional capacity (Aune, 1976).

A possible significant correlation with differences in tissue-specific aging rates in an organism would be ^{corresponding} tissue differences in DNA repair capability. The ability of any tissue to monitor and repair DNA damage depends not only on the extent, nature, and location of the induced lesion(s) (Hart and Trosko, 1976; Trosko and Hart, 1976) but also on the proliferative state of the cellular population (i.e., replicating, quiescent, or postmitotic) under examination. Postmitotic cells not only lack postreplication repair but also exhibit a significant decrease in excision repair capability when compared with proliferating cells from the same organism. This difference in DNA repair levels suggests that since postmitotic tissues should accumulate more lesions with time than proliferating tissues, they should also age at a faster rate. This is, in fact, supported by aging studies in vivo, suggesting that a positive correlation does exist between DNA repair capability and lifespan.

If this correlation is to be considered a strong one, then agents which induce extensive DNA damage should accelerate the aging process. Early investigators did, in fact, conclude that exposure to ionizing radiation accelerated the appearance of normal aging parameters. However, more recent extensive reassessments of the accumulated data have suggested

both quantitative and qualitative differences from the normal aging process (Walburg, 1975). It is apparent that exposure to ionizing radiation primarily increases the incidence of neoplasia resulting in premature death and shortened lifespans. Investigations based on exposure to chemical mutagens have been equally negative in observing an acceleration of normal aging (Alexander and Connell, 1960; Curtis, 1963; Stevenson and Curtis, 1971). These findings suggest that DNA damage is not fundamental to the aging process. However, it is possible that aging is normally a consequence of changes in a relatively few critical tissues and large exposures to DNA damaging agents mask these important alterations.

Of equal importance to a correlation between lifespan and DNA repair capability is the expression of accelerated aging in genetic syndromes with demonstrable decreases in DNA repair capability. Unfortunately, the findings so far have been largely inconclusive. Neither ataxia telangiectasia nor Fanconi's anemia, both DNA repair deficiency syndromes, express any significant signs of premature aging. Only xeroderma pigmentosum exhibits a possible correlation between the level of repair deficiency and increased signs of accelerated aging. The more severe the loss in excision repair capability, the greater the indications of premature aging of the skin and neural tissues (Robbins et al., 1974).

In a similar fashion, syndromes considered to express accelerated aging components (e.g., progeria and Werner's syndrome) have not been demonstrated to involve DNA repair deficiencies. Progeria has been suggested to involve a defect in DNA strand break rejoining capability (Epstein et al., 1973a) but subsequent investigation demonstrated that normal repair levels existed among some progeric patients (Epstein et al., 1974; Regan and Setlow, 1974; Bradley et al., 1976). Possibly the repair loss

in some progeric cell lines was a result of gene repression and/or altered metabolism but not due to an intrinsic defect in DNA repair (Little, 1976).

This lack of a consistent correlation between DNA repair deficiency and premature aging suggests that the two processes are not necessarily related. However, one of the difficulties in making that conclusion is that it assumes that all DNA repair processes are equally important. This is obviously not true since individuals affected with the variant form of xeroderma pigmentosum involving a deficiency in post-replication repair exhibit milder clinical manifestations than the excision repair deficient form (Cleaver and Bootsma, 1975). This might be expected since postmitotic cells have no need for that particular repair process.

The strongest experimental finding in support of a direct correlation between lifespan and DNA repair capability is the observation of Hart and Setlow (1973) that fibroblast cell lines derived from longer-lived animals show greater levels of UV-induced UDS. However, this correlation has not been confirmed within the primates (H. Kato, personal communication).
more
Further, since aging does not appear to be a consequence of a decline in cellular systems capable of proliferation, the validity of this correlation

must be questioned. It has been proposed that lifespan is controlled by small groups of essential postmitotic cells in critical organs and tissues such as in the brain or neuroendocrine system (Franks, 1974). If this is the case, then a comparison of DNA repair capability in these cells between different species is of primary importance.

Aging does not appear to be a direct consequence of a decline in DNA repair capability. The possibility remains that differences in lifespan among the various animal species could be a direct result of different

levels of DNA repair capability. However, current experimental support of this hypothesis remains inconclusive. Also it has yet to be proven that unrepaired DNA lesions accumulate with age in some of the tissues more critical to aging. While it is expected that DNA damage certainly contributes to the aging process, it is difficult to conclude that all of aging is a consequence of their accumulation. Other factors may play an equally critical role, such as the degree of genetic redundancy for vital functions within the genome (Hart and Trosko, 1976).

This review should be no means discourage research directed at the possible relationship between DNA repair and aging. The current state of understanding concerning both of these processes is still in its infancy. This review should, rather, indicate the areas of interest in which research should be encouraged due to either presently conflicting results and/or a lack of information. Extensive investigations into the relationships between DNA repair and aging can only advance our understanding of the underlying molecular processes inherent to both systems.

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