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Mice, Myths, and Men

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Mr. President, Ann, Warren and Joy, Shirley, members of the Council, and Guests. It is a cliché to say that it is a great honor, but cliché or not, it is just that to give this lecture honoring Lauriston Taylor. It is a great disappointment that you, Dr. and Mrs. Taylor, are not here. Laurie, for many years, I, like many others, have enjoyed your sharp twinkling mind, your good sense and verve, and we so envy your ability to defy time. I hope that you, Charlie, will convey to Dr. and Mrs. Taylor all our best wishes.

The surprise and pleasure in joining the distinguished roster of those chosen to give a Lauriston Taylor lecture was tempered with concern. I was sitting in the Committee Room at NCRP attending a meeting on the question of extrapolation across species, facing the photographs of the 17 illustrious Taylor lecturers, when the president informed me. The realization of the enormity of the task of approaching the standard previously set by those looking at me from the frames on the wall was immediate. I have been fortunate to have known 16 of the 17 previous lecturers, and it is a great honor to join them.

Last year, I had the honor of introducing Warren Sinclair as the Seventeenth Lauriston Taylor Lecturer. Because of the pact we made, my introduction was brief and really did not do justice to his multi-faceted career. It is the lecturer's prerogative to select the person to introduce his talk. It was natural that I should select Warren. Shirley and I have had the fortune to be friends of Joy and Warren for nearly 35 years. I am very grateful to you, Warren, not only for your more than generous introduction, but also for all those years of friendship and support in your various roles, not the least of which was Director of the Division of Biology and Medicine at Argonne National Laboratory.

On the surface, the subject of my talk has little to do with the subject of this meeting, "Extremely-Low-Frequency Electromagnetic Fields," but perhaps it is not all that unsuitable to have a person who comes from the land of leprechauns speak at a meeting devoted to ELFs.

Introduction

I am going to discuss some examples of how different experimental animal systems have helped to answer questions about the effects of radiation, in particular, carcinogenesis, and to indicate how the new experimental model systems promise an even more exciting future. Entwined in these themes will be observations about susceptibility and extrapolation across species. The hope of developing acceptable methods of extrapolation of estimates of the risk of radiogenic cancer increases as molecular biology reveals the trail of remarkable similarities in the genetic control of many functions common to many species.

First, a few words relative to the title of this talk. There is no trouble in recognizing mice, and usually no trouble in recognizing men, but how about myths? The definition of myth is: "an explanation that is partially or wholly fictitious" (1). Under this broad umbrella, even a considerable number of the current opinions about certain aspects of cancer might qualify. Biology is rife with dogmas, and many a dogma has a good chance of becoming a myth in its old age. I am not going to spend much time on myths. You will no doubt spot some as we go along and perhaps even in some of the dogmatic statements that I make.

Alexander Pope knew of what he spoke when he wrote in 1773, "The proper study of mankind is man, a being darkly wise and rudely great with too much knowledge for the skeptic side with too much weakness for the stoic's pride. . ." (2). The studies of the effects of radiation on humans have been a remarkable endeavor. While much of the emphasis has been on the estimate of risks for radiation protection purposes, of equal if not greater importance is their role in revealing the nature of cancer. The investment into the elucidation of the risks posed by radiation and how the deposition of energy leads to the gallimaufry of effects at the molecular and cellular level, as well as on the whole organism, has been large, but so has the return on that investment.

Despite the intensive efforts in epidemiological studies, it is unlikely that all the relevant questions can be answered by such studies alone, for example, the stochastic effects of neutrons, protons, and heavy ions, and the influence of dose rate and protraction. Experimental animal studies have roles in the quest for answers to these questions and about mechanisms. It was not many years ago that the differences between humans and experimental animals were considered so great that the value

of what could be learned about cancer in humans from experiments on mice was thought by many to be little. There has been a remarkable change in that attitude as the genetics of human diseases and corresponding models have become understood.

Homology

Superficially, the two gentle folk shown in Figure 1 do not appear to have all that much in common, but appearances are deceiving. The homology of DNA from these two primates is estimated to be about 98%. That is a humbling but an exciting fact. Imagine the use that Clarence Darrow would have made of that knowledge in the Scopes trial. While many genes may be similar in different species, many are located on different chromosomes or at different loci. It can be seen in Fig. 2 that, in the case of the Y chromosome of the human and the chimpanzee, many of the genes are at comparable locations (3).

The conservation of genes across the phylla that molecular biology continues to reveal is remarkable. The similarity of the DNA sequences in the so-called homeobox genes involved in development, between the fruit fly, mouse, and human, is striking - such continuity with such diversity. The homology between the DNA of the human and the mouse is not so marked as between primates although is perhaps about 70%. What is important for cancer research is the marked conservation of the genes involved in the processes that are important to the development of cancer.

In Figure 3 are shown some of the genes on chromosome 18 of the mouse, and on the left are listed

the human chromosomes on which the homologous genes are found (4). I chose mouse chromosome 18 because the Min mutation (officially APC^{Min}) is located on it. This gene is the homolog of the human adenomatous polyposis coli gene that plays a role in certain cancers of the colon, but more about that later.

A major concern about even attempting to extrapolate estimates of risks of radiation-induced cancer across species has been that the mechanisms of carcinogenesis were so different among different species that it would negate the validity of extrapolation. The more that has become known about the genes involved in cancer, especially those related to the initial events in carcinogenesis, the more have the reasons for considering methods of extrapolation across species increased.

Mouse Model Systems

The conservation of genes has made experimental mammalian and other systems valid model systems for addressing many biological questions, but one can also make use of the quirks of evolution. For many years, my colleague Ron Ley and I searched for a suitable and manageable experimental system to investigate the question of what was the lesion that ultraviolet radiation (UVR) induced in DNA that initiated the events that could proceed to cancer of the skin. The circumstantial evidence that DNA damage, and pyrimidine dimers in particular, was involved in the induction of skin cancer by UVR was considerable. For example, the similarity in the action spectra for the interaction of pyrimidine dimers and neoplastic transformation *in vitro* suggested a causal relationship (5). The marked susceptibility of patients with xeroderma pigmentosum who develop

skin cancer as a result of exposure to sunlight also suggests a causal relationship between UVR-induced pyrimidine dimers and cancer. However, nondimer damage induced by UVR such as the pyrimidine (6-4) pyrimidone photoproduct could not be ruled out as the lesion of importance. The way to answer the question was to make use of the fact that the cells of certain organisms, including marsupials, were able to repair DNA damage by photoreactivation. Photoreactivation involves an enzyme photolyase which binds specifically to pyrimidine dimers. Exposure of the photolyase-dimer complex with light (300-500 nm) and absorption of a photon converts the dimerized pyrimidines to the monomeric form (6). The search was for a beast that possessed the ability to repair cyclobutane pyrimidine dimers by photoreactivation (Fig. 4). Humans and rodents do not produce photoreactivating enzyme at levels capable of repairing the damage to DNA induced by UVR, marsupials do. While it would have been fun to have a Wallaby about, there were some obvious drawbacks. Frogs and fishes, both of which do possess photoreactivating enzyme, were not for us. Eventually we obtained a manageable-sized opossum, *Monodelphis domestica,* and Ley et al. (7) carried out the experiments that took advantage of the repair process shown in Fig. 4; the results are indicated in Fig. 5. You can see that multiple exposures to UVR (280-400 nm with a peak at 313 nm) are very effective in the production of carcinomas of the skin and that exposure to photoreactivating light (320-700 nm) after each exposure to UVR reduced the subsequent incidence of carcinomas markedly. It is probable that skin cancers were not eliminated completely because not all the pyrimidine dimers were removed. This was not the first experiment to use a model system that was based on photoreactivation, a specific repair mechanism of cyclobutane pyrimidine dimers, but it was the first to provide what we hoped was unequivocal evidence of the role of the dimers as the initial lesion in skin carcinogenesis. Setlow and colleagues (9) used *Poecilia formosa,*

a fish known as the Amazon molly (which I think was at the suggestion of J.D. Regan). They transplanted cells from various organs of the fish after exposing one group of cells to single exposures of UVR (254 nm) and one to UVR followed by photoreactivating light. In this innovative experiment they found "exuberant growth" of thyroid cells that they interpreted as tumors, but because the fish were clones, and thus the cells expressed no antigens by which the transplanted cells could be distinguished from the host's cells, the source of the proliferative cells could not be confirmed. Furthermore, thyroid tissue is distributed more widely in fish than in mammals, making the identification of growths that originated from the transplanted cells difficult.

One must be cautious in the interpretation of the experiments with *Monodelphis* that appear to indict pyrimidine dimers as the culprit in the initiation of skin cancer. It is possible that photoreactivating the UVR-induced pyrimidine dimers reduces the demand on repair enzymes which in turn results in more excision repair of non-dimer photoproducts such as the 6-4 photoproduct.

The point to be made from the results of these experiments in relation to the discussion of the use of experimental animals in cancer research is that judicious choice of animals that differ from humans can be as rewarding as searching for similarities.

Susceptibility for Cancer

An understanding of the factors that determine susceptibility for cancer in general and in particular the cancers induced by either ionizing or ultraviolet radiation is central to understanding the

mechanism of carcinogenesis and has importance for radiation protection.

The lifestyles, diets, and the habit of some humans to smoke tobacco confound the determination of the precise role of the genetic makeup in the mechanism for cancer. The data in Tables 1 and 2 illustrate the contrast in the small range of total cancer incidences among countries with the very large variation of incidences of specific types of cancer between countries.

TABLE 1
Variations in the Incidence of Common Cancer
(Doll, 1977) (10)

Variation in the incidence of specific types between countries	Variation in the total cancer incidence between countries
up to ~200	~2

TABLE 2
Range of Cancer Mortality in Various Countries
Cumulative Risk: Males and Females 35-74 y of age (Coleman et al., 1993) (11)

Cancer Site	High-Incidence Population	Ratio High:Low	Low-Incidence Population
Esophagus	Hungary	23	Finland
Stomach	Japan	8	USA
Colon	Czechoslovakia	9	Greece
Lung	Hungary	7	Finland
Prostate	Norway	12	Japan

The variation in the incidences of individual types of cancer is related to differences in lifestyle, diet, and exposure to environmental factors that in turn are related to ethnic and geographical differences. An important question is how does the background rate of specific cancers, which are probably due

to a combination of genetic and extrinsic factors, influence the susceptibility for induction of cancer by radiation. A further question is how much of the susceptibility is determined by inherited factors. The change in the use of absolute risks to relative risks in estimates for risk of radiogenic cancer for radiation purposes is a recognition of the influence of the background rate. The question of background rates of cancers enters the practical problem of the appropriate use and transfer of risk estimates from one population to another. This is a problem to which Charles Land and Warren Sinclair have given a great deal of thought (12). Consider the estimates of risk for induction of cancer of the gastrointestinal track. From the risk coefficient for individual sites that were used by ICRP (13) to derive the total lifetime risk of excess cancer mortality (Table 3), it can be seen that 45% of the total cancer risk is attributed to cancers of the gastrointestinal tract.

TABLE 3
Probability of radiation-induced cancer
at specific sites in general population (ICRP 1991)

Organ or Tissue	Probability of fatal cancer (10^{-2} Sv^{-1})		
Bladder	0.30		
Bone Marrow	0.50		
Bone Surface	0.05		
Breast	0.20		
Liver	0.15		
Lung	0.85		
Ovary	0.10		
Skin	0.02		
Thyroid	0.08		
Esophagus	0.30	}	
Stomach	1.00	}	=2.25
Colon	0.85	}	
Remainder	0.50		
TOTAL	5.00		

Is it reasonable to believe that the contribution of cancers of the gut to the total risk of cancer induced by radiation in the U.S. population is so high? There is a major difference in the background rates of cancers of the gut between the U.S. and Japanese populations. In the U.S. these cancers account for about 15% of all cancer mortality, in contrast to 30% for the Japanese. None of the studies of populations in the western world indicate that the risk of radiation-induced cancer of the gut is as high as suggested by the data from atomic bomb survivors (14). In the case of the stomach, the study of Griem et al. (15) suggests that the risk might be a factor of 16 times less in the U.S. population than in the Japanese. There is a need for studies of the risk of induction of cancers of the gastrointestinal tract in western populations. In rodents the gastrointestinal tract appears to be one of the systems that is resistant to the induction of cancer by radiation.

Differences in Susceptibility for Cancer

There may be large differences in the susceptibility for cancer among species other than mice, but not many species have been studied in the detail necessary to determine the susceptibility for the induction of cancer by radiation. At one end of the spectrum of susceptibility for the effects of radiation and for cancer is the burro. This beast is resistant not only to the exhortations of man but also to manmade radiation. In Fig. 6, the results of an experiment are shown in which the animals outlived the scientific lives of some of the investigators. Fortunately Lushbaugh and his colleagues persisted and collected and collated the data shown in Fig. 6 (16). The number of animals is not large but is sufficient to indicate the remarkable resistance to radiation and what appears to be

unusually low incidences of cancer in both unirradiated and irradiated animals. One wonders with envy what sort of tumor suppressor genes this animal must have. The burro is certainly not an animal that comes to mind when embarking on a cancer research project, but there must be some intriguing information about susceptibility to cancer to be found from a study of their cells.

Laboratory mice are maintained under a regimen to which only a Trappist monk might aspire. Unattractive as the never-changing diet may be, it allows the investigation of the role of the genetic background in the susceptibility for specific cancers and their induction. In the case of solid cancers, the susceptibility for radiation induction appears to be related to the incidence of the naturally occurring tumors (17). This relationship appears to be true also for chemical carcinogenesis of the liver (18). In Figure 7, it can be seen that the rates of both naturally occurring and radiation-induced lung cancers are greater in the BALB/c strain than in C57BL/6 mice. An extreme of this relationship was found in BCF_1 mice, where no mammary carcinomas were found in either the unirradiated controls or mice exposed to various low doses of neutrons (20). This apparent relationship of susceptibility for radiation induction to the background rate supports the choice of relative risk models. It should be noted that it is thought that the background rate of breast cancer in humans does not influence the response to radiation and the absolute risk model is the one of choice (21).

An interesting example of the relationship of the incidence of naturally occurring and radiation-induced cancers is chronic myeloid leukemia in the atomic bomb survivors. This type of leukemia is more common in the survivors at Hiroshima than those at Nagasaki (22). In fact the difference between Hiroshima and Nagasaki was so marked that Robin Mole referred to the leukemias in the

Hiroshima survivors as neutron dependent (23). That was before the new dosimetry, DS86, and when the contribution of neutrons to the dose incurred by the survivors at Hiroshima was thought to be significant. Even if DS86 is not completely accurate, the idea that the differential in the rates of radiation-induced myeloid leukemia between Hiroshima and Nagasaki is due to the difference in the neutron component of the exposures at the two cities is not likely to be the explanation. First of all, the background rate of chronic myeloid leukemia is also higher in Hiroshima than Nagasaki (22). This illustrates not only the dependence of leukemia induction on the background rate, but significant regional differences in the background rates of a specific type of cancer. Second, in mice myeloid leukemia can be induced by neutrons, X rays or gamma rays. The difference in the effect is quantitative, not qualitative, and the rate of both naturally occurring and induced leukemias is dependent on age, sex, and the strain of mouse, but not on radiation quality. The idea that a specific cancer might be associated with a specific carcinogenic agent or radiation quality was ahead of its time and will be discussed later. The search for a link between specific mutations and specific causative agents is being pursued now with vigor.

The reasons for difference in susceptibility lie of course in the inherited characteristics. But almost all the research in this area has concentrated on defective repair and germline mutations, which are factors that influence the probability of initiation of the carcinogenic process being induced. But there is good reason to believe that it is the factors that influence the expression of the initial events that are the important determinants of whether a cancer occurs in the lifetime of the person exposed. One can look at the opposite side of the coin of expression, namely suppression. Tissues clearly differ in their ability to suppress the expression of initiated cells and thus to abrogate the full

potential for producing overt cancer. Most of you who are my age have initiated cells that are effectively suppressed. It was thought by many that initiation was a relatively rare event even after irradiation. Various studies would suggest that this is a myth. The data from a study of Ullrich (24), shown in Fig. 8, illustrate this point, and Mulcahy et al. (25) have quantified the initiated cells in the induction of thyroid cancer and shown how many more cells are initiated than become tumors. The experiment shown in Fig. 8 also illustrates how effective tissues are in suppressing the expression of the potential for development into a cancer. There is a large differential in the number of initiated cells that have the potential of developing into a cancer and the number that express that potential if they remain in their community of cells in an intact tissue. For the potential for malignant growth to be expressed, the communal restraints must be lost. As can be seen from Fig. 8, the dispersion of the cells and a favorable environment for growth are all that is required. This evidence that a tissue can suppress expression of the potential for malignant growth would have pleased Virchow. Virchow, sometimes called the father of pathology, discussed diseases in terms of social science. In the case of the cancer, the community of cells that constitute a tissue can often handle errant members, but if the community is disrupted, for example by considerable cell killing, or when the tissue is excised and cells are separated for culture, then the malignant members of the cell community can escape suppression. Recently there has been an interest in the elimination of neoplastic or potentially neoplastic cells, in particular by apoptosis, and whether this could explain differences in the incidence of cancer among tissues (26).

Susceptibility: Expression of Initiation

As noted above, most of the studies of susceptibility have concentrated on inherent factors influencing initiation such as DNA repair. However, the experiments on tissues such as skin and breast indicate the importance of the expression of the cells with the potential for developing into a malignancy in determining the incidence of cancer. Not much is known about the role of genetic factors that influence expression.

In an experiment investigating skin cancer induction by exposure to psoralen plus UVA (320-400 nm), a combination that is known as PUVA and is used widely in the treatment of psoriasis, we were able to establish differences in the expression of the lesions in DNA, psoralen-DNA crosslinks, in two different strains of mice. The process of the formation of psoralen-DNA crosslinks is shown in Fig. 9. In these experiments we used 8-methoxypsoralen, which intercalates with DNA but does not bind to it unless exposure to the appropriate wavelengths of UV radiation occurs. With exposure to UVA, a monoadduct involving a pyrimidine base on one DNA strand is formed, and this is converted to the diadduct or psoralen-DNA crosslink with the further absorption of UVA. These psoralen-DNA crosslinks, which are the lesions that initiate carcinogenesis, can be assayed with considerable accuracy (28). We exposed two strains of hairless mice to induce the same load of psoralen-DNA links in the epidermal cells. The incidence of carcinomas induced in the skin was markedly different in the two strains of mice (Fig. 10), but the difference disappeared when expression of the induced lesions was "promoted" by treatment with 12-O-tetradeconyl-phorbol-13-acetate (29). Clearly the strain-dependent differences in cancer susceptibility were due to genetic

factors influencing expression. Despite considerable effort, the underlying mechanism responsible for the difference in expression or suppression of the initiated cells between the two strains has not been identified. The only difference between the strains found so far is in the growth rate of the mice. The SKH-hr-1 mice grow more rapidly and are significantly heavier than the HRS/J/An1 mice as adults. It is the SKH-hr-1 mice that are the more susceptible. It is possible that factors that influence somatic growth also affect the growth and rate of appearance of the squamous cell carcinomas. Obesity has been associated with early appearance of tumors in mice. It is clear that genetic factors influence the events involved in expression and that an elucidation of these factors is just as important to the understanding of susceptibility as is the knowledge about the initiating events.

In the case of solid cancers, the latent period between exposure to radiation and the clinical detection of a cancer is long, in some cases greater than 20 years, and little is known about what is going on during that long interval. For example, how are the phenotypic characteristics of a neoplastically transformed cell maintained but their expression suppressed? In certain types of radiogenic cancer, the age at which the excess incidence is detectable appears to be independent of the age at exposure but dependent on the age that the naturally occurring cancers of the same type start to increase. In other words, latent periods are variable and the length is related to some processes that are related to age.

Models of Carcinogenesis

Perhaps the most pervading dogma contained in the descriptions and modeling of carcinogenesis is that carcinogenesis is a multistage process. The implication is that there are distinct events that may be separated in time. The concept that carcinogenesis is a progressive process is almost 60 years old. In 1935, Rous and Beard (30) described the progression of papillomas to carcinomas, and Green (31) described the development of naturally occurring mammary cancer in rabbits in terms of "progressive steps in a graded evolutionary process." These studies, and in particular those of Berenblum and Shubik (32), led to the acceptance of a two-stage model for skin carcinogenesis. It was Foulds who collated the evidence, concluded it supported the concept of progression and suggested that "the basic idea of progression is the same as epigenetic development in embryology" (33). Since then more stages have been added, but most importantly, the opportunity to attempt a correlation between the changes at the cellular and tissue level with molecular events has come with the application of the techniques of molecular biology.

Two models of the progressive development of malignancy that stem from very different studies are illustrated in Figs. 11 and 12. Barrett's model (Fig. 11) is based on the studies of Syrian hamster embryo cells in culture (34). A major advantage of the use of these cells is that they are primary diploid cells and therefore the important change, transformation, can be studied. Cells such as C3H 10T1/2 cells are transformed, that is they appear to be immortalized, having attained unlimited proliferative capacity, and are aneuploid. Both the embryonic hamster cells that grow to form the colonies and C3H 10T1/2 cells are fibroblasts, which are not the cells of origin of carcinomas. The

3model proposed by Barrett is based on the suggestion that the proliferative capacity of a particular cell escapes the fate of senescence and lives to divide at will and awry. It was Hayflick, based on observations of primary cultures, who suggested that stem cells senesced after a limited number of divisions (35). If this was true *in vivo*, it would mean that the stem cells of renewal systems, for example, the hematopoietic system and the gut, would have to have a hierarchial organization of stem cells. Such an arrangement would be quite feasible in the bone marrow, where the spatial arrangement of stem cells is of much less consequence than in the gut with its individual crypt population. Such a design for the intestine seems less likely.

The *in vitro* Syrian hamster cell system makes it possible to study the changes in primary cells after exposure to a carcinogenic agent. The first phenotypic change is in the morphology of the cell colonies. The community of cells that make up the colonies no longer show order, discrete edges and a smoothness, but are disorganized with the cells growing in a criss-cross pattern. Nobody knows what are the underlying molecular changes responsible for the morphological changes. The cells in most of these morphologically altered colonies senesce, but some become what has become known as immortalized. This change may occur spontaneously in rodents and is the earmark of the transformation from a primary cell strain to a cell line. It is this change that is so preciously rare in human cells, rarely occurring either spontaneously or after exposure to radiation at rates that make it easy for the investigator to detect. A cardinal change that is associated with immortalization is the change from a diploid state to an aneuploid state. In Syrian hamster cells, the change often involves trisomy of chromosome 11 (36).

Immortalization is said to be a multistep process involving both activation and inactivation of genes, in particular the genes associated with cellular senescence. The model which has been applied to the question of chemical carcinogenesis suggests at least two more stages of genetic changes are required for the cell to become malignant. These changes are thought to involve an activation of an oncogene, and in addition, an inactivation of an as yet unidentified suppressor gene.

The model suggested by Vogelstein and his colleagues to describe the carcinogenic process in colorectal tissues is very different and is shown in Figure 12 (37). The model is based on the belief that both the clinical and histopathological evidence suggest that colorectal carcinomas develop from benign lesions. The model takes into account the changes at the tissue, chromosomal and gene levels, and since the data come from human tumors environmental, dietary and inherited factors are all involved. The sequential nature of the histological changes from adenoma to carcinoma are put forward with confidence (38). Corresponding sequential changes at the molecular level are much less certain, but what is considered important is the accumulation of the various mutations. The mutations involve both oncogenes and tumor suppressor genes. Roughly speaking, tumors that show the greatest number of the characteristics of malignancy show the largest number of mutations, which is perhaps good evidence of progressive development. Mutation in a member of the *ras* family of oncogenes is a relatively early change and thus can be found in adenomas. Deletions of alleles on chromosomes 5, 17 and 18 have been found in colorectal tumors. At least three tumor suppressor genes, *deleted in colon cancer (DCC), adenomatous polyposis coli (APC)* and *p53,* are involved in the tumorigenesis. As is the case in many types of solid cancers, loss of alleles of chromosome 17p is a very common finding in the malignant colorectal tumors. The region lost

contains the *p53* gene and mutations in the remaining alleles of *p53* occur, resulting in the loss of a functioning tumor suppressor gene. This change involving *p53* is a common finding in many human solid cancers. The cells and tissues of malignant tumors differ markedly in morphology, behavior and gene expression from their normal counterparts, and the relevant documentation is accumulating at a pace that is hard to keep up with.

There are two aspects of the models that deserve thought. First, how can a single brief exposure to a low dose of radiation result in perhaps six or more mutations at different loci? Such a number of mutations is consistent with the interpretation of Armitage and Doll that the incidence of cancers in adult humans increases with the sixth power of age (39). That all the mutations could be caused by a single low dose of radiation is, to say the least, improbable. Similarly, the probability of this number of mutations occurring independently in the development of a naturally occurring cancer without some marked change in mutation rate is equally improbable. The information about mutation rates in normal human somatic cells is woefully sparse, as only about three loci have been studied adequately. But rates of $1.9 \pm 0.5 \times 10^{-7}$ and $2.2 \pm 0.3 \times 10^{-7}$ have been reported for *hgprt* and *tk* loci in cells of a human diploid lymphoblast line (40). Mutation rates at this locus in other cells, such as human diploid fibroblasts, are also in the range of 10^{-6} to 10^{-7} and indicate the improbability of five or six sequential and independent mutations. There are at least a couple of possible explanations. Nowell suggested in 1976 that tumor progression resulted from genetic instability and clonal selection, each mutation endowing a selective growth advantage (41). The evidence of instability of the genome of cells involved in tumor development has accumulated in the last few years. Another possible explanation for the multiple mutations is that mutations in the genes

responsible for the stability of the genome, and the fidelity of replication, including the correct location of genes (42-44), may occur, resulting in what has been referred to as a mutator phenotype (45). The discovery of mutations of *hMSH2*, the mismatch repair gene, in cases of hereditary non-nonpolyposis colorectal cancer (45) is supporting evidence that an induced mutation can result in instability and a mutator phenotype. A second gene which encodes the protein *hMLH1*, so called because it is the homolog of the bacterial DNA mismatch repair protein MutL, has been reported recently. This is yet another example of the conservation of genes essential to functions that are common across the phylla. There are a number of genes in *Escherichia coli* that cope with the repair of mismatched DNA, including *mutL*. If any of these genes are defective, there is an increase in the spontaneous mutation rate. The evidence is that genomic instability of simple repeated sequences is an early event in colorectal tumorigenesis and accounts for an increase in mutation rates by perhaps as much as a factor of 10 (46). There is evidence that radiation can induce increases in delayed mutations (47) and chromosomal instability (48-51). But whether such changes can account for the induction by a single small dose of radiation of the number of mutations required for cancer is not clear.

In the study of cancer induction, it is important to remember that there is more than one pathway in the development of cancers. While obviously at some point in carcinogenesis a common pathway is reached, the early events may differ between cell types and tissues. Differences in the penetrance of mutations among tissues, the role of imprinting and the occurrence of fragile sites on chromosomes may all play a part in susceptibility. In the models of progression of tumors, the dogma is that malignant tumors develop from benign tumors. For example, it is generally claimed

that squamous cell carcinomas of the skin evolve from papillomas.

This may be so, but it is impossible to dismiss the possibility that malignant tumors such as carcinomas can develop independently from benign neoplasms, such as adenomas, and not sequentially. We have found that, in three different strains of hairless mice exposed to UV radiation, there was no correlation between the number of papillomas and the final incidence of squamous cell carcinomas. In one strain, papillomas were seen rarely but squamous cell carcinomas were as frequent as in other strains. Hyperplastic nodules and adenomas are the common early outcome after exposure of the liver to chemical carcinogens; however, microtumors consisting of cells with a malignant phenotype can be found at the same time the benign neoplasias are starting to appear. It is possible that progression occurs so rapidly that the stages cannot be recognized (52). It has been reported that cancers induced in the large intestine of the rat do not always go through a typical adenomatous stage (53).

If carcinogenesis is a multistage process, does radiation influence all the stages? Many years ago Armitage and Doll, as noted above, suggested that the increasing incidence of cancer with age was consistent with a process in which about six events were required (39). Considering the long latent period and the number of stages, it would not seem unreasonable if with increasing age there was an increasing number of cells in the later stages of progression to cancer. If this were the case, one might expect susceptibility to increase with age. An important question is whether susceptibility is related to the number of stages that the cells at risk have undergone. In Fig. 13, a potential paradox is illustrated, namely, that as the probability of naturally occurring cancer increases with age,

susceptibility for the induction of solid cancers by radiation decreases with age. This suggests that irradiation of cells that are at some stage along the pathway to cancer has little or no influence on progression. However, some experimental evidence suggests that radiation acts in a manner similar to promoters, but it is difficult to sort out interactions that are involved in these observations from susceptibility. The fact that a single brief exposure to radiation is a complete carcinogen suggests that the initial lesion can result in the initiated cells traversing all the stages involved in carcinogenesis. Nevertheless, the evidence suggests that the prominent effect of radiation is on initiation (54).

As the mechanisms of carcinogenesis are revealed and more fully understood, the opinion of what are the rate-limiting steps in the development of a cancer may alter. The three changes that must occur for a cancer that is life-threatening to develop are: (1) Altered control of cell proliferation resulting in excess cell production. It is often assumed that immortalization is a necessary change; however, the difficulty of getting cells from some human tumors to continue dividing *in vitro* suggests that immortalization may not be a mandatory change in all cases. (2) Angiogenesis is essential if a tumor is to grow beyond the size sustainable by diffusion of oxygen and nutrients, a mere 1-2 mm^3, or about 10^6 cells. The concept that tumor growth is dependent on angiogenesis was proposed by Folkman in 1971 (55), and he and his colleagues have described the induction of angiogenesis during the transition from hyperplasia to neoplasia (56). It should be noted that angiogenesis does not necessarily correlate with the degree of malignancy; some benign tumors can be just as angiogenic as malignancies, and some tumor cells can avoid the need for neovascularization by growing in thin sheets as can occur in tissues of the nervous system. (3) The

development of the ability of the tumor to invade neighboring tissues and to metastasize. This involves the expression of genes that are normally suppressed.

It is not clear what the precise relationship of angiogenesis and the ability to invade to the initial events is, but there appears to be a correlation between angiogenesis and the development of the metastatic phenotype (57). The roles of growth factors in initiating angiogenesis and tumor suppressor genes are being elucidated, and the interference with angiogenesis as a potential therapy remains an attractive possibility.

The Cell Cycle and Cancer

The mutations shown in the illustrations of the models of carcinogenesis are ones that affect the control of cell proliferation and in the main by the loss of the products that check the progression of cells at certain points in the cell cycle. The understanding of how the cell cycle and proliferation are controlled has progressed at an increasingly rapid rate in the last 40 years. The identification of the S phase by Alma Howard and Stephen Pelc (58) by ^{32}P labeling made it possible to distinguish four phases of the cycle instead of just interphase and mitosis (Fig. 14, left panel). The phases G_1 and G_2 were so named to indicate not only the gaps in time, but the gaps in information about what went on during those phases. The beautifully simple experiment carried out by Howard and Pelc stimulated a great deal of research into what was happening in the two "gaps," especially the syntheses required before cells start replicating DNA and for the assembly of the mitotic apparatus. These syntheses must be completed before the objective of the cell cycle, namely, cell proliferation,

can be accomplished.

Now, as you can see from the right-hand side panel of Fig. 14, you need an instruction book to figure out how all the checks and balances on progression of cells through the cycle work. Much of the understanding about the genes and their products, such as the cyclins and their cyclin-dependent kinases (CDKs) which are integrators of the signals involving growth factors that drive the cell cycle (59) come from studies of yeast. This is yet another striking example of the conservation of genes that are concerned with a process central to growth and development. There are a number of proteins, p16, p17, p21, that block the cyclins and act as brakes on the progression of cells across checkpoints. It is at the cycle of the cell that growth factors, growth-inhibitory factors, oncogenes and tumor suppressor genes meet to exert their influence on the delicate balance of cell production, so vital for homeostasis and so deadly when it goes awry (60). A major function of two of the tumor suppressor genes, *Rb* and *p53*, is to limit progression. The check on progression into mitosis is more rigid than the control of the entry of cells into DNA synthesis, which is the reason that parenchymal cells such as liver cells increase their ploidy with age. In any tissue that retains the ability to proliferate into adult life, if only to respond to injury, the cells have a very low but positive probability of entering the S phase and a lower probability of progressing to mitosis; thus with age cell populations become hyperdiploid.

Perhaps the most singular change associated with neoplasia is altered control of cell proliferation - cell production exceeds cell loss. I say altered control advisedly; although it is often said that malignancy involves the loss of control, complete loss of control is a rare event in neoplasms of solid

tissues. However, the inability to control cell proliferation may become more marked as a tumor becomes more malignant.

As indicated above, there is increasing interest in the role of selective elimination of cells with a damaged genome. The *p53* tumor suppressor gene produces a protein that blocks cyclins and thus causes delay in the progression of cells through the cycle, which allows time for repair. The same gene can also produce the proteins that lead to apoptosis. Mutations in the *p53* gene are, so far, the most common genetic changes related to cancer, and these may be point mutations, allelic loss, rearrangements and deletions of the gene. Most importantly, inherited mutations in *p53* cause predisposition to cancer (61, 62) and explain the Li-Fraumeni Syndrome (63). When a null mutation was introduced into the *p53* gene by homologous recombination into murine embryonic stem cells, mice developed apparently normally but had an increased susceptibility for tumors which appeared by 6 months of age (64). The variety of tumors reminded the investigators of the Li-Fraumeni Syndrome.

Since this lecture was given, the understanding of the role of *p53* and a number of genes in control of cell proliferation and in carcinogenesis has continued to accumulate at such a rate that it is becoming difficult to keep informed. Of particular importance to radiation carcinogenesis is the report (65) that *p53*-deficient mice are extremely susceptible to radiation-induced tumorigenesis. It was found that radiation decreased the latent period of tumors in mice made heterozygous for *p53* using the gene "knockout" technique. It was also found that the genetic alterations in the remaining wild-type allele in the tumors arising in the irradiated mice were different from those found in the

naturally occurring tumors. This difference suggests that the *p53* gene may have been the target. It should be possible to confirm the probability of such a hit from dose-response relationships, the size of the gene and assumptions about the alteration of the allele. As more and more is revealed about the *p53* gene, the somewhat flamboyant title "guardian of the genome" (66) seems a less fanciful description of the gene. It is clearly a guardian of progression through the cell cycle, a power in the success of DNA repair and an arbiter of division or death by apoptosis - and an influence on malignant progression (67) - quite some gene.

It is repeatedly claimed that the path to better risk estimates of radiation-induced cancer is the understanding of mechanisms. Certainly an identification of the targets for radiation-induced cancer, their number and size, and whether repair can occur when they are damaged could improve the understanding of dose-response relationships.

Experimental Animal Model Systems

A few decades ago a small group of workers, in particular Walter Heston, used mice to reveal the genetic aspects of cancer, for example, the relationship between susceptibility to induced pulmonary tumors and certain known genes in mice (68). There are marked strain-dependent differences in the probability of both naturally occurring and induced lung tumors. Using conventional crosses, Heston showed that more than one locus influenced susceptibility. If two strains have the same alleles at all but one locus, it can appear that susceptibility behaves as a single Mendelian gene. Thirty years ago Bloom and Falconer suggested there was a major recessive gene in C57BL mice that they named "pulmonary tumor resistance" (*ptr*), and that it accounted for three quarters of the

difference in susceptibility between the resistant C57BL and susceptible A strain (69). The rest of the difference was attributed to minor genes. More recently it has been suggested that susceptibility to the induction of lung tumors by urethan can be accounted for by three genes (70). In liver, a single locus named "hepato-carcinogen sensitivity" (*hcs*) has been reported to be responsible for about 85% of the difference in susceptibility between the resistant C57BL/6J and susceptible C3H/He/J mice (71). The identification of specific genes that control susceptibility to and development of tumors in rodents is proving to be a valuable approach to understanding carcinogenesis. The application of the new techniques should allow the nature and function of these genes in the mechanism of carcinogenesis to be elucidated. In Table 4 are shown a few examples of rodent tumors that are being used to study mechanisms and the underlying genetic aspects.

Table 4
Experimental Animal Model Systems

Tumors	Species	Genetic Role	Reference
Small and Large Intestine	Mouse	*Min* mutation predisposes to intestinal tumors	Moser et al. (72)
		Mom-1 modifies the expression of the *Min* mutation	Dietrich et al. (73)
Mammary Tumors	Rat	Predisposition of *Mcs* gene and other loci	Gould and Zhang (74)
Renal Cell Carcinoma	Rat	Susceptibility gene for predisposition to renal cell carcinoma	Eker and Mossige (75) Walker et al. (76)
Liver Tumors	Mouse	Genetic control of predisposition to hepatocarcinogenesis	Drinkwater and Ginsler (71)
	Mouse	Mutational activation of c-Ha-*ras* and susceptibility	Buchmann et al. (77)
Myeloid Leukemia	Mouse	Specific deletion in Chromosome 2	Hayata et al. (78) Brecken et al. (79)
		Role of telomeric repeat sequences in predisposition to myeloid leukemia	Silver and Cox (80)

I cannot discuss in detail these exciting experimental model systems, but I would draw attention to the potential value of the new mutation, *Min*, found in mice that predisposes to multiple intestinal neoplasia and which is homologous to the adenomatous polyposis coli gene, *Apc*, in humans (72) and also to the *Mom-1* gene, which modifies the expression of the tumors found in the *Min1*

phenotype (73). These mice should prove very valuable in elucidating the role of radiation in the induction of cancer of the gut. Such information could improve the risk estimates for gut, which are a major determinant of the total risk of radiogenic cancer.

Not only does the researcher have this battery of mouse model systems, but now he can "manufacture" mice tailored to answering specific questions. A number of questions can now be answered by the techniques that allow manipulation of the mouse's genome, either by introducing DNA constructs to produce transgenic mice that will overexpress a particular gene (81) or by using embryonic cells to develop a mouse that cannot express a specific gene, the so-called "knockout" mouse (82). An example of this latter technique was discussed in the segment about the role of *p53* in carcinogenesis.

Extrapolation of Risk across Species

The more that is understood about the genomes of different species, the more remarkable is the evidence that the genes important for controlling cell replication and DNA repair are conserved. The maintenance of the fidelity of the genome, whether by what is known as housekeeping or by repair of more complex damage, is central to the avoidance of naturally occurring cancer. The changes involved in carcinogenesis are much more similar in humans and rodents than they are different. Considering all the genetic evidence, the hope of developing acceptable methods of extrapolation of risk across species does not seem an empty one. If Jacques Monod could say that an elephant is like an *E. coli* only more so, it shouldn't be impossible to span the differences between mouse and

man and to extrapolate risks of radiation-induced cancer. Sufficient data are available for radiogenic cancer in both humans and mice; thus any method of extrapolation can be tested immediately. The task is not without its pitfalls, one of which is a mixture of semantics and pathogenesis (an odd couple in itself). For example, tumors of the lung in both humans and mice are analyzed under the category "lung tumors," yet there are a number of different cell types giving rise to cancers so categorized. In mice lung tumors arise from type II alveolar cells or Clara cells, whereas in humans the origin may not be only those cell types but also neuroendocrine cells, giving rise to the so-called small cell lung cancer. Squamous cell carcinomas are common in humans, but very rare in mice. The distinction between tumors of different cell types may become important if their susceptibility and therefore the dose-response relationships of the induction of tumors arising from these different cells are different. The pathogenesis becomes important if the mechanisms, especially in the expression of initiated cells, are different. For example, the hormonal influences in mammary carcinogenesis are different between humans and mice. So those wishing to extrapolate must proceed judiciously, but they should proceed.

A further perceived problem is the great disparity in the time that development of a cancer after exposure takes. However, if the relative toxicity (83) or a relative risk approach (84) is used, this disparity can be overcome for solid cancers.

Despite the reluctance to use life shortening as an index of radiation effects, a good case can be made for it. Consider the following: all or almost all of the life shortening due to low doses and protracted irradiation can be accounted for by the excess cancers induced by the radiation. Life shortening is

an integrated effect of the different types of tumors and can be determined accurately and economically. The life spans of different strains of mice vary from the low 300's (days) to the high 900's, but the percentage of life shortening relative to the life span of the control populations is remarkably similar (85). In Table 5, a comparison of the radiation-induced life shortening resulting from protracted exposure to low-LET radiation of mice and humans is shown.

Table 5
Radiation-induced life shortening

	Mouse[a]	Human[b]	
Duration of exposure (days)	688	17,630	(18-65 years)
Loss of life span/10 mSv (days)	0.2	3.9	
Life span of unexposed populations (days)	997	25,550	(70 years)
Percentage of life span lost	0.02	0.015	

[a] Estimate from data of Grahn, Thomson, and Carnes (86)
[b] Data from BEIR V, NAS/NRC (87)

The induced life shortening relative to the control life spans is remarkably similar for the two species. While this approach is not the final answer, it is an encouraging step.

Another potential use of life shortening is that a single value for a low-dose and dose-rate effectiveness factor and a single RBE for neutron radiation could be obtained. NRCP SC-1-4 is currently preparing a report on extrapolation of risk across species.

Susceptible Subpopulations

The understanding of the underlying genetics of susceptibility is of importance not only for

understanding of the mechanisms of carcinogenesis, but also for radiation protection standards. Radiation protection standards are set on the assumption that there is a normal distribution of susceptibility for radiation-induced stochastic effects and that radiation limits should protect the most susceptible members of the population. However, it is necessary to keep a close eye on the burgeoning information about susceptibility to ensure that sensitive subpopulations are not at unacceptable risk, and that the risks are not overestimated for the rest of the population.

Most cancers are caused by somatic mutations, and their occurrence is sporadic and unpredictable, but a proportion of cancers occur in individuals who have an inherited susceptibility due to a germline mutation. For radiation protection, the questions are: What proportion of the population has an increased susceptibility, and are they susceptible to the induction of cancer by radiation? The frequency of any one of the conditions due to a germline mutation is low, and the contribution of these diseases to the total cancer incidence appears to be small. However, Bodmer considers that 20% of all cancers may be associated with an inherited susceptibility (88). Perhaps more important is whether these inherited conditions confer susceptibility for radiogenic cancers and in particular whether the heterozygotes are susceptible. Complete answers to these questions are not available. In the case of the Basal Cell Nevus Syndrome, patients are very susceptible to the induction of basal cell carcinomas by X rays; for many of the syndromes, the answer is not so clear-cut. An interesting example of what is likely to turn out to be a small susceptible subpopulation has been reported (89). In the study of the atomic bomb survivors, Tokuanga and his coworkers identified a number of women exposed before the age of 20 who developed breast cancer before the age of 35 (Fig. 15). The excess relative risk was about six times greater than in women with the same cancer at later

ages. The risk of breast cancer before the age of 35 is known to occur in the Li-Fraumeni Syndrome (63), in which there is a germline mutation in the tumor suppressor gene *p53*. The cases of early-onset breast cancer that occurred in the atomic bomb survivors do not have this syndrome. It seems likely that the gene involved is one of the *BRCA* genes. One of these genes, *BRCA1* on chromosome 17, accounts for the inherited susceptibility in those patients with multiple breast cancers and ovarian cancers and about 50% of the cases with early-onset breast cancer (90). The fraction of the atomic survivors with breast cancer who showed early onset is small but should stimulate careful examination of other familial cancers to determine the susceptible subpopulations more accurately.

Specific Radiation-induced Mutations

The ability of epidemiological studies to detect excess risk of cancer as a result of exposure to low doses of radiation, particularly protracted exposures, is limited by the statistical power that can be attained with low-frequency events and the limits on the size of populations that can be studied (91). The identification of a number of genes, such as *p53*, that are involved in many cancers and the difference in the probability of deletions and point mutations being induced by different carcinogenic agents have raised hopes of being able to decide if a specific tumor was induced by radiation. The *p53* gene appears just the gene for investigating whether the spectra of mutations can help in the identification of the carcinogen that induced a specific tumor (92). A very large fraction of the mutations in the *p53* gene are missense mutations that result in the amino acids that can be detected in *p53* protein. Analysis of the mutations has shown that changes in the codons of the *p53* gene depend on the cancer site. In cancers of the lung and the liver G:C to T:A transversions are common,

but in other types of solid cancers, mutations of A:T base pairs are predominant. The hope is that these different mutations reflect the mechanisms which in turn are related to the causative agent. There is clear-cut evidence of the involvement of UVR in skin cancer (93); Brash et al. have identified CC-TT double base changes in *p53*. Such a change is known to be caused by UVR. This finding makes it possible to clinch the belief that UVR interacts with ionizing radiation in the production of skin cancer in patients exposed to ionizing radiation (94). For example, the identification of mutations in *p53* at dipyrimidine sites in the DNA of tumors of the face or neck of patients with tinea capitis and treated with X rays would provide the proof of the role of UVR in the genesis of these tumors.

Profiles of Radiation-induced Cancer

Despite the rapid advances in the molecular biological aspects of carcinogenesis, it will be some time before an understanding of the mechanisms will improve risk estimates of cancer induction by low doses of radiation. Some years ago the probability of causation was introduced to help the courts decide whether a specific cancer had been induced by a prior exposure to radiation (95). The estimate of the probability was based on dose, age at exposure, gender and cancer site. The dogma has been that you cannot distinguish a cancer caused by radiation from one caused by other agents. The hope is that the identification of specific radiation-induced mutations in tumors will turn this dogma into a myth, but much remains to prove the potential of this approach.

No use has been made of information about the cancer site or the type of cancer. For example, a

hemangiosarcoma of the liver is unlikely to be caused by radiation but likely to have been caused by vinyl chloride. A carcinoma of the mastoid is well nigh certain to have been caused by radium. These are not common tumors, you say, and that is so. However, even in the case of the common cancer of the lung it has been shown that small cell cancers are associated with exposures to radon (96) and squamous cell carcinomas with smoking (97). Also, Land et al. reported that radiogenic lung cancers are much more likely to be small cell carcinomas than adenocarcinomas (98). As more information accumulates about cancers and specific markers, it should be possible to prepare a profile of any specific tumor and decide whether radiation was the causative agent, at least with more certainty than with the probability of causation.

Conclusion

I have discussed the use of different strains of mice to investigate susceptibility for the induction of cancers and some of the factors influencing susceptibility, in particular the expression of tumors. The selection of an opossum with the ability to photoreactivate pyrimidine dimers induced by UVR made it possible to establish the role of such dimers in the induction of skin cancer.

Evolution has moulded man in a very different form from mouse. However slim the theme of this talk may have been, I hope that there has been a thread woven into this partial canvas depicting carcinogenesis, and that thread is the remarkable conservation among species of the genes vital to the maintenance of the genome, its repair, and cell proliferation and differentiation. Many of these genes are involved in the process of carcinogenesis. Although there are large biological differences

between man and mouse, the homologous nature of so many of the salient genes in these species makes the mouse an excellent experimental model system for the study of carcinogenesis. The ability to induce overexpression of genes and to knockout genes in the mouse almost at will has expanded the possibilities of investigating the mechanisms of carcinogenesis by various agents, especially radiation, enormously.

The validity of methods of extrapolation of risk estimates across species is being critically evaluated, and it is suggested that the use of radiation-induced life shortening in mice after exposure to small doses or protracted irradiation is justified in estimating RBEs that could be considered in the selection of radiation weighting factors, W_Rs and also in the selection of DDREFs.

The potential impact of susceptible subpopulations on the estimates of risk must be examined as the information about the nature of inherited susceptibility becomes better known. Lastly, the new approaches for the identification of the causative agent of specific tumors are promising but as yet do not contribute any improvement to the estimates of the effects of low doses. Nevertheless, a more comprehensive approach to the determination of whether a cancer was caused by radiation is worthwhile.

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Figure Legends

Fig. 1 Left Panel: Albert Einstein; Right Panel: a young chimpanzee

Fig. 2 Some of the genes located on the Y chromosomes of the human and the chimpanzee. Adapted from O'Brien and Graves (3).

Fig. 3 Homology of human and murine genes. The numbers shown on the schematic refer to the human chromosomes on which the genes mapped on Chromosome 18 of the mouse are located. The figure has been adapted from Copeland et al. (4).

Fig. 4 Schematic of the repair of pyrimidine dimers by photoreactivation. The figure has been adapted from Hanawalt (8).

Fig. 5 Probability of skin tumors in *Monodelphis domestica* as a function of time (weeks) after first exposure to UVR (280-400 nm): o—o, UVR followed by exposure to 320-700 nm light: ●—●, and to 320-700 nm light alone: □—□. The figure has been adapted from Ley et al. (7).

Fig. 6 Kaplan-Meier survivor function estimates for burros exposed to gamma rays, gamma rays and neutrons, and for unexposed controls plotted as a function of time (years) to death after exposure. The figure has been adapted from Lushbaugh et al. (16).

Fig. 7 Mortality rate in BALB/c: ●—●, and C57BL/6 mice with lung cancer as a function of dose (Gy). Data from (17) and reproduced with permission from Fry and Carnes (19).

Fig. 8. Schematic showing the incidence of breast cancer (14%) after a single dose (100 rad) of γ rays and that all of the irradiated mice contained mammary cells that produced tumors when transplanted to hosts. Data from (24).

Fig. 9 Schematic of the formation of psoralen-DNA crosslinks with treatment with 8-methoxypsoralen and UVA (300-400 nm) known as PUVA.

Fig. 10 Percentage of squamous cell carcinomas in SKH (o—o) and HRS (●- - -●) hairless mice after exposure to PUVA, with and without subsequent treatment with 12-O-tetradeconyl-phorbol-13-acetate as a function of time (weeks) after first exposure. Reproduced with permission (29).

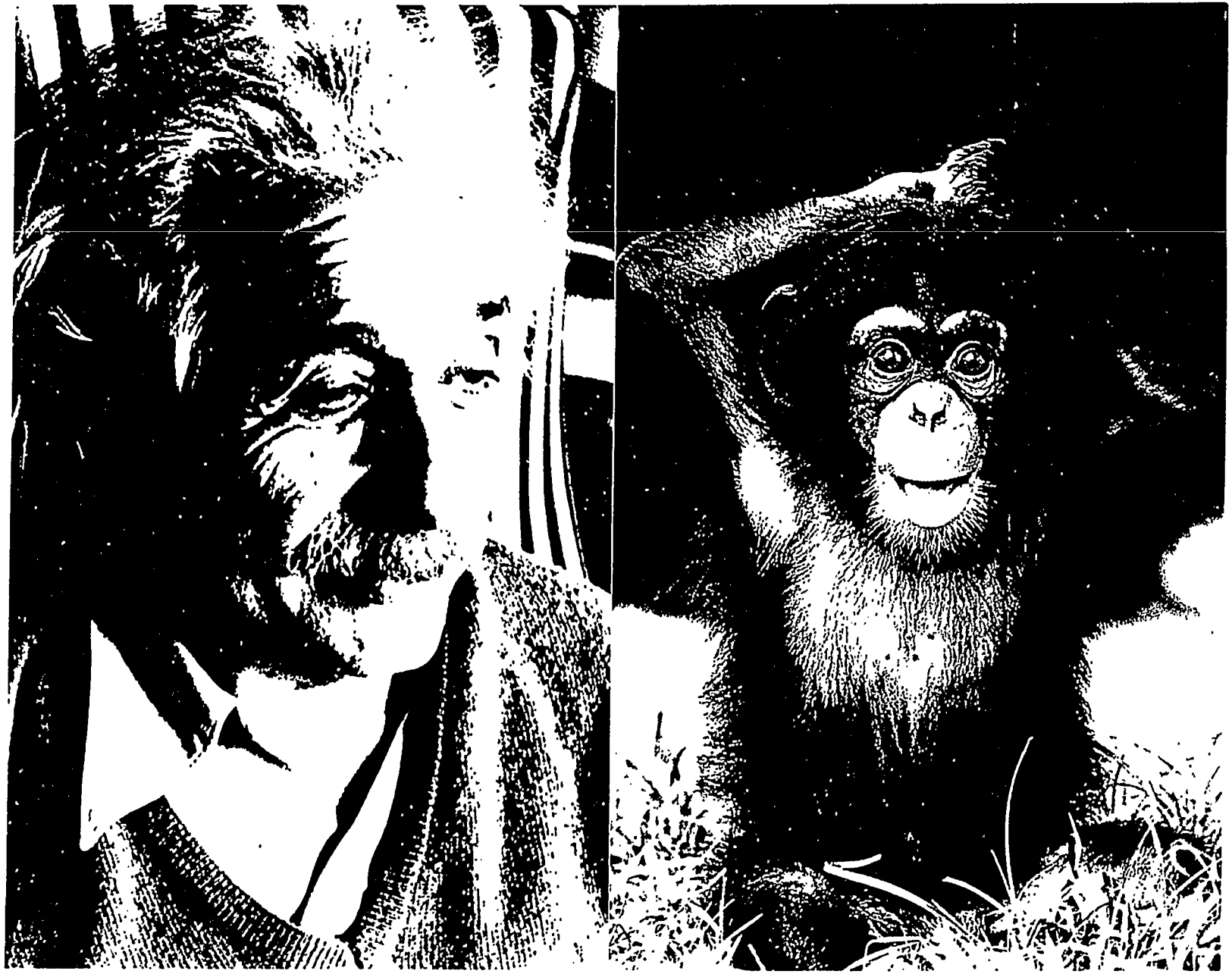
Fig. 11 Schematic of oncogenic transformation of Syrian hamster cells. Reproduced with permission from Barrett (34).

Fig. 12 Schematic of colon carcinogenesis. Reproduced with permission from Fearon and Vogelstein (37).

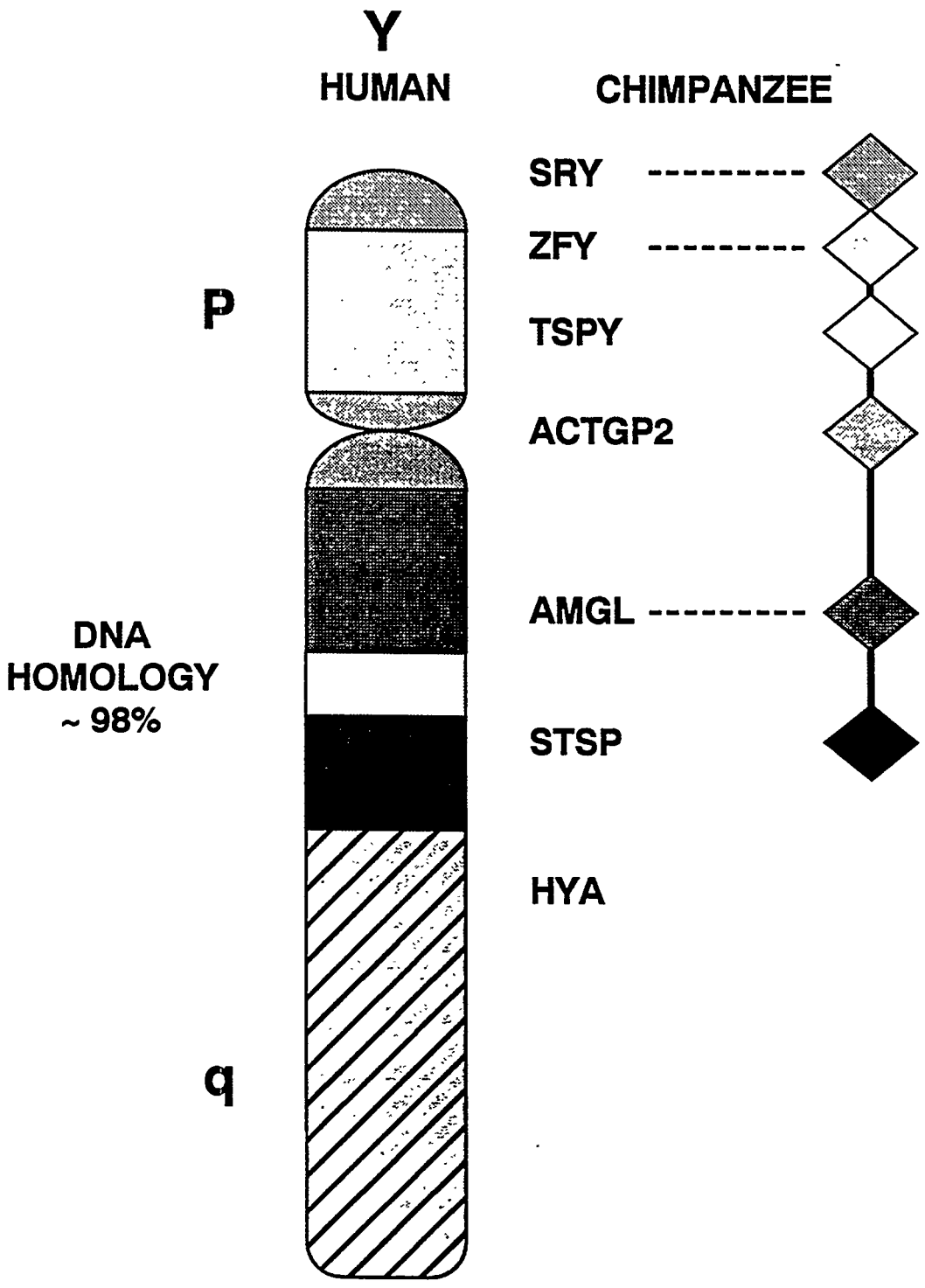
Fig. 13 Schematic of multistage carcinogenesis.

Fig. 14 The cell cycle as seen in 1953 (58) and 1993 (from various sources including [59, 60]). The schematic shows some of the many factors that influence cell proliferation. There are those, such as the cyclins, that ensure the progress of cells through the cell cycle (one or more cyclins may block differentiation). The level of the cyclins is cyclical and they act by controlling the cyclin-dependent kinases (CDKs). The products of genes, such as retinoblastoma (*Rb*) and *p53* and transforming growth factor β (TGF-β), which blocks the synthesis of one of the CDKs, play different roles in applying the brakes to the progression of cells from one phase of the cell cycle to another. A number of other factors, such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), fibroblast growth factor (FGF) and insulin growth factor (IGF1), are included. The comparison of the schematic representation of the cell cycle as seen in 1953 and forty years later indicates the progress that has been made in a study that continues. The relationship of the changes, in some cases by mutation, in the factors influencing the cell cycle and cancer is a major focus of these studies.

Fig. 15 Estimated excess relative risk per Sv, by interval of attained age (25-34, 35-44, 45-54, 55-64, 65-74 and ≥75), with fitted model ERR $(D;A) = \alpha D \cdot \exp(\beta_2 A)$, where D is equivalent dose in Sv (neutron RBE = 10) and A is attained age. Estimates and 90% confidence limits stratified on city, age at time of the bombings, attained age and period. Reproduced with permission from Land et al. (98). Total number of cases appears above the upper confidence limit for each interval of attained age (89).



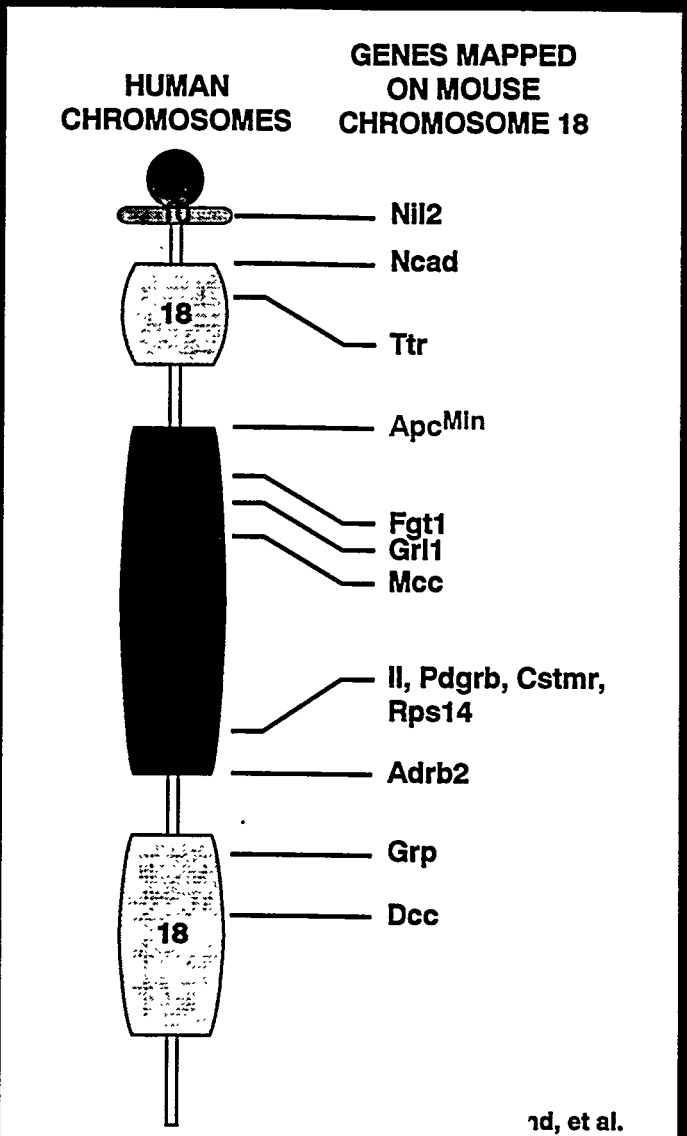
Conservation of Genes



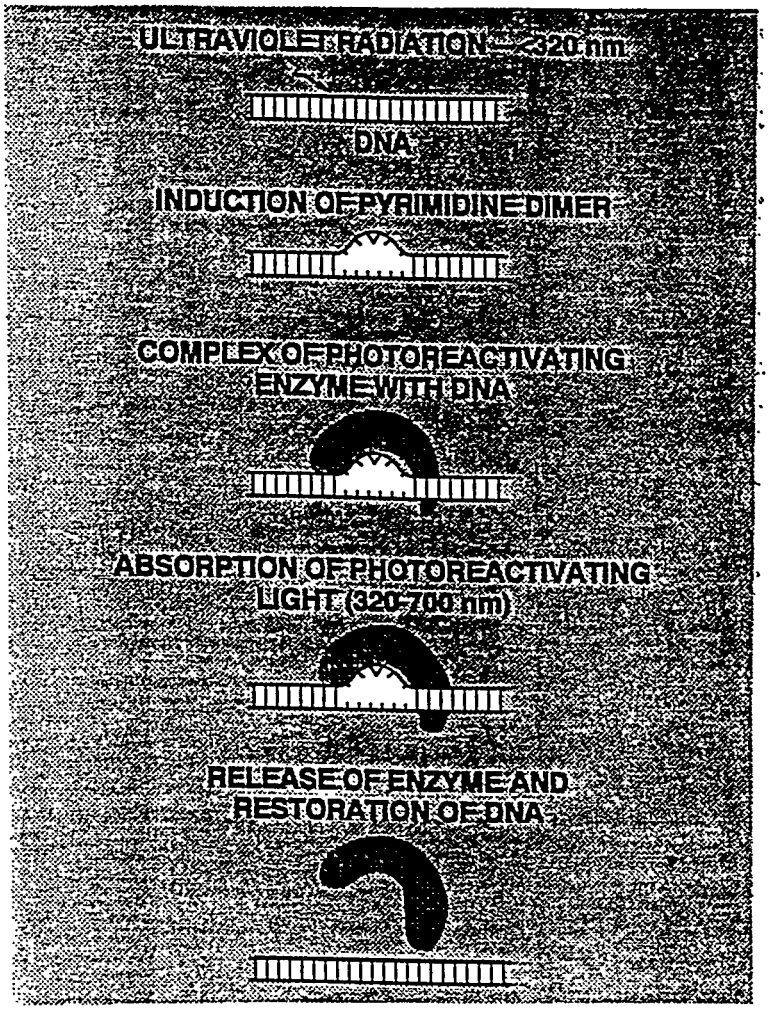
From O'Brien and Marshall Graves (1991)

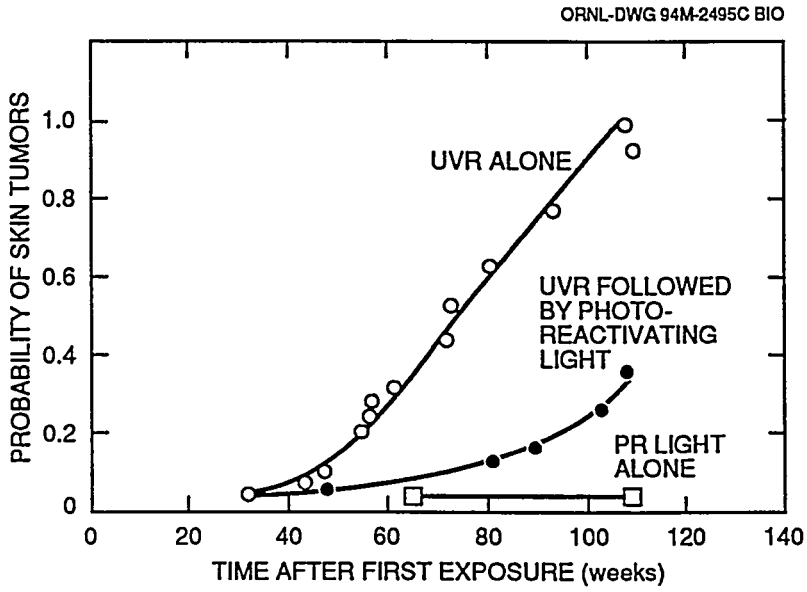
ORNL-DWG 94M-2519C BIO

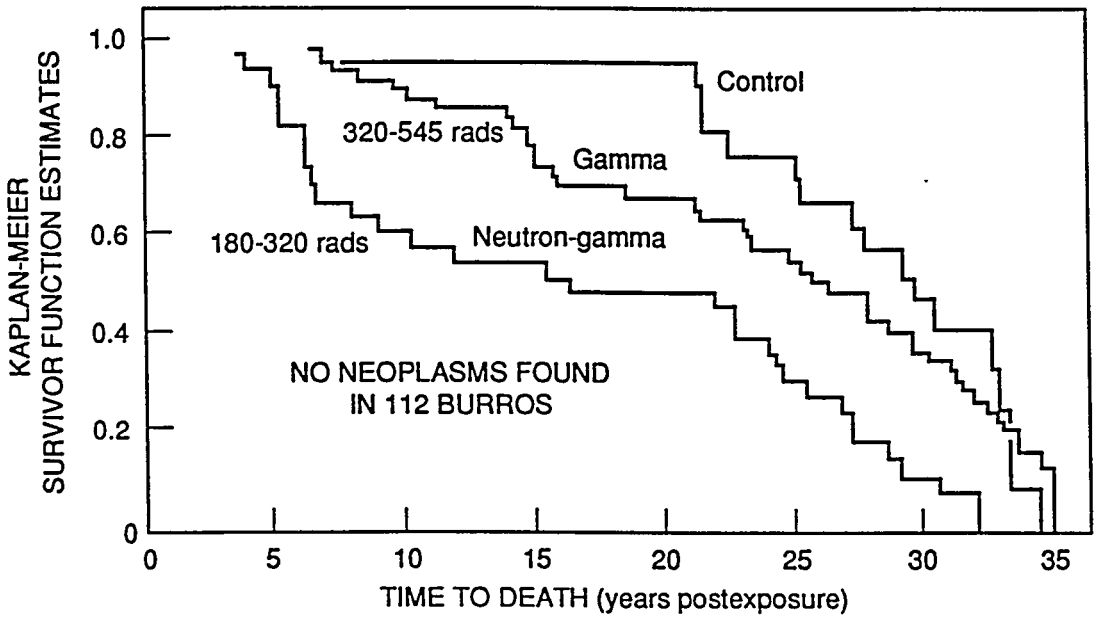
Homology

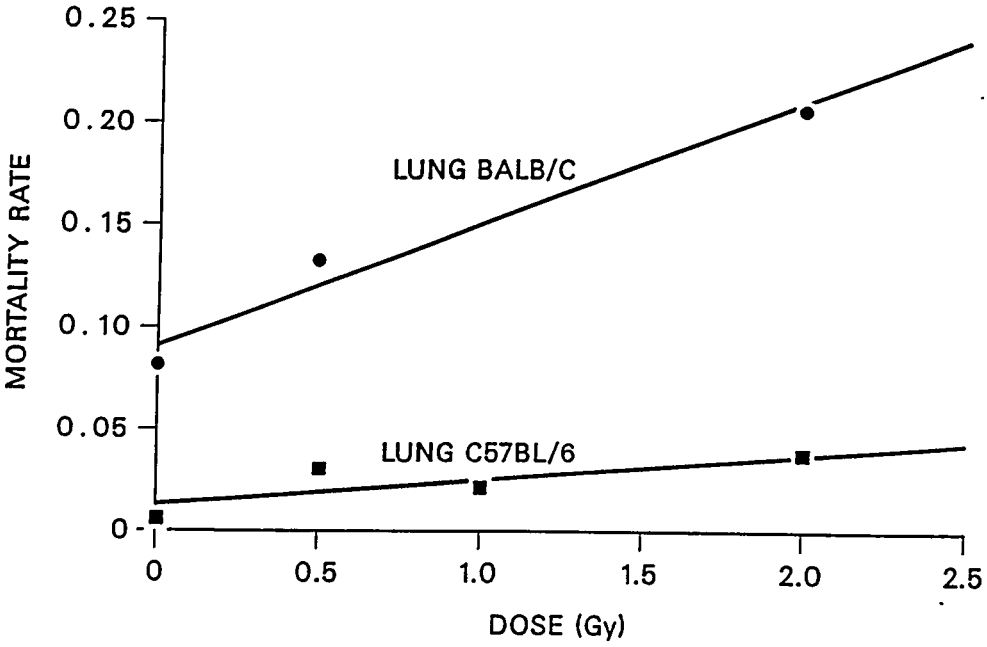


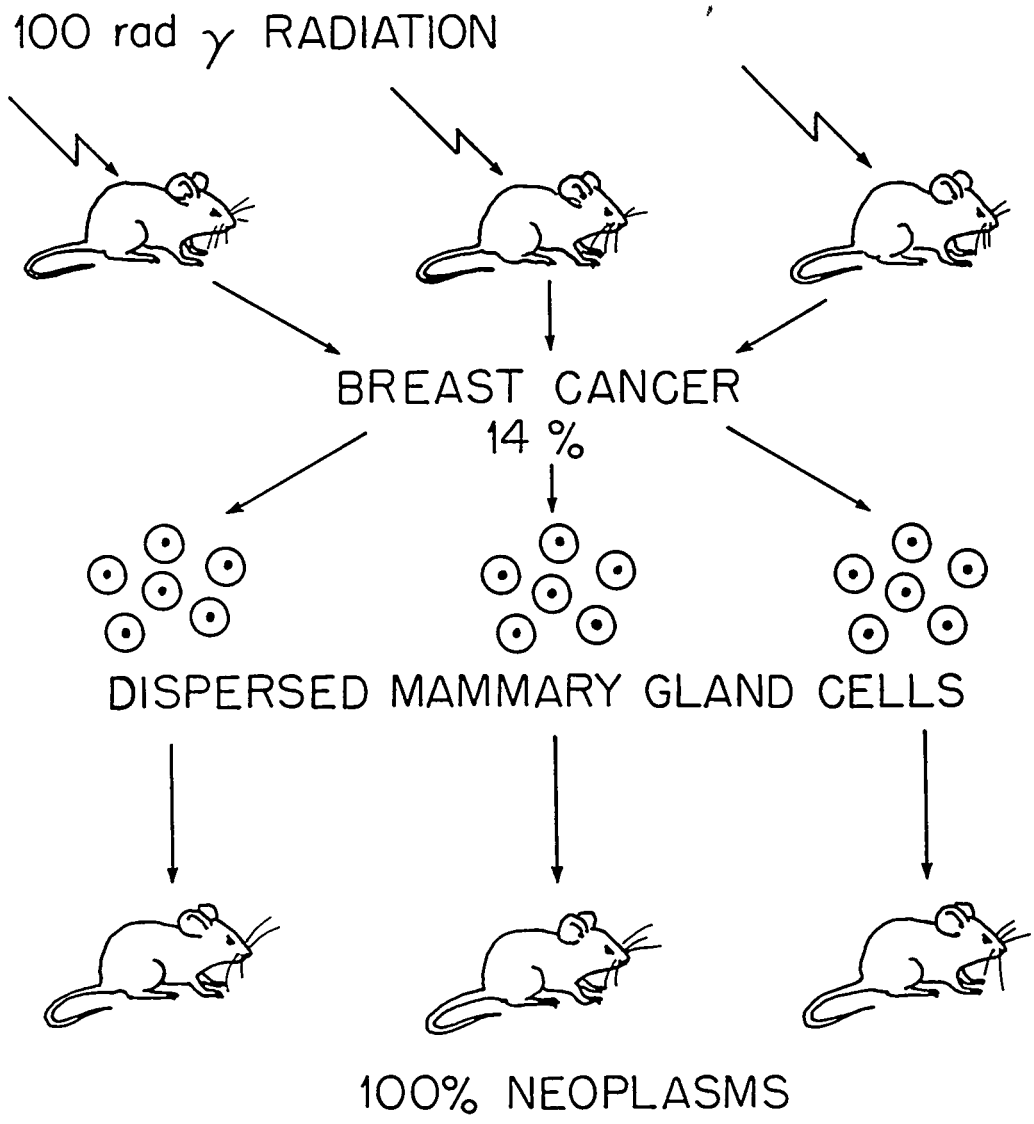
ORNL-DWG 94M-2518C BIO


ULTRAVIOLET RADIATION <320 nm
DNA
INDUCTION OF PYRIMIDINE DIMER
COMPLEX OF PHOTOREACTIVATING ENZYME WITH DNA
ABSORPTION OF PHOTOREACTIVATING LIGHT (320-700 nm)
RELEASE OF ENZYME AND RESTORATION OF DNA


ORNL-DWG 94M-2495C BIO
PROBABILITY OF SKIN TUMORS
1.0
0.8
0.6
0.4
0.2
0
UVR ALONE
UVR FOLLOWED BY PHOTO-REACTIVATING LIGHT
PR LIGHT ALONE
0
20
40
60
80
100
120
140
TIME AFTER FIRST EXPOSURE (weeks)

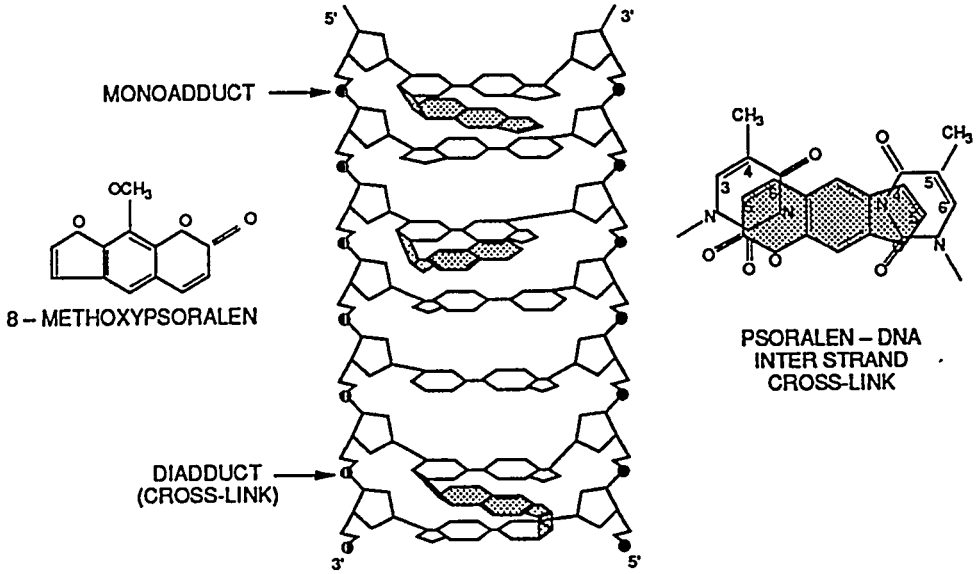

KAPLAN-MEIER
SURVIVOR FUNCTION ESTIMATES
1.0
0.8
0.6
0.4
0.2
0
0
5
10
15
20
25
30
35
TIME TO DEATH (years postexposure)
Control
320-545 rads
Gamma
180-320 rads
Neutron-gamma
NO NEOPLASMS FOUND
IN 112 BURROS


0.25
0.20
0.15
0.10
0.05
0
MORTALITY RATE
LUNG BALB/C
LUNG C57BL/6
0
0.5
1.0
1.5
2.0
2.5
DOSE (Gy)

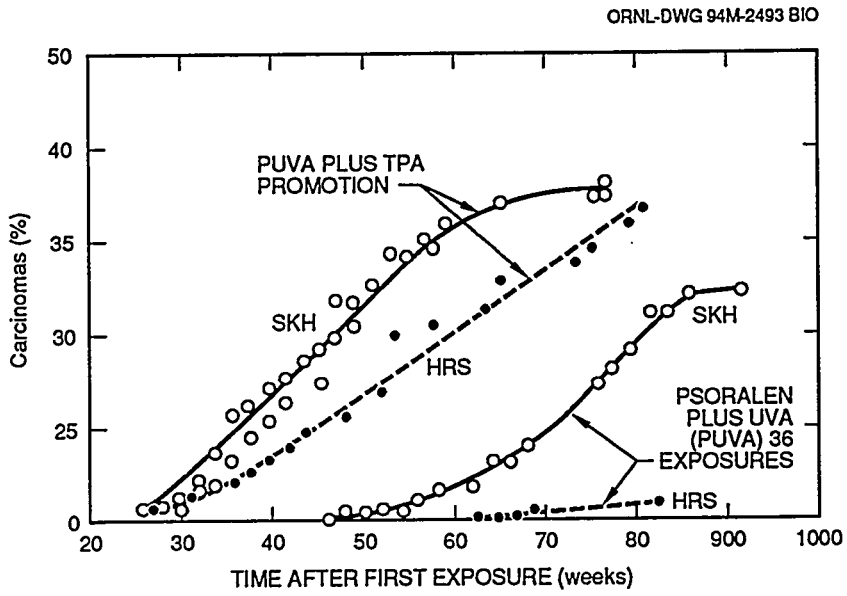


Data from Ullrich

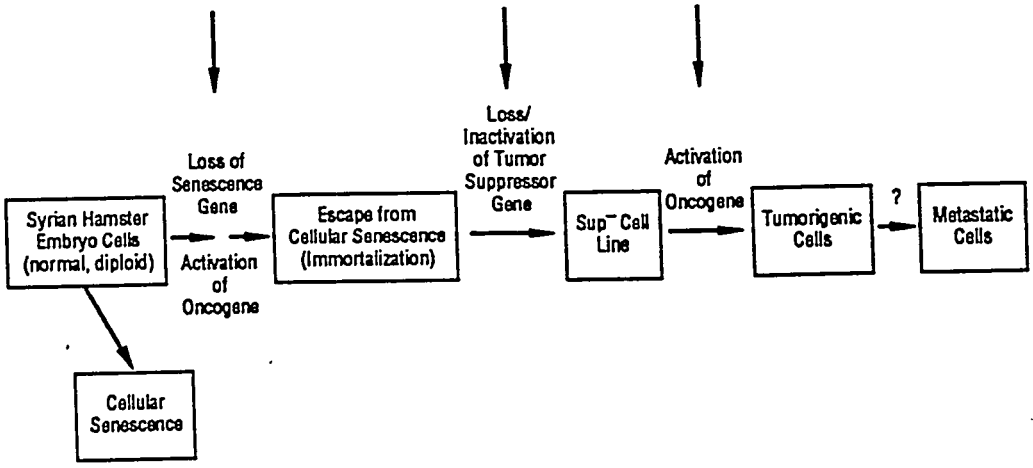
ORNL-DWG 94M-2503 BIO



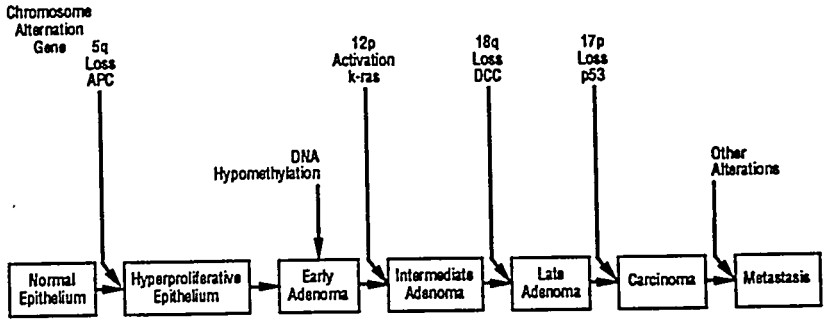
PSORALEN – DNA CROSS-LINKS CAN BE QUANTIFIED


ORNL-DWG 94M-2493 BIO
50
40
35
30
25
0
Carcinomas (%)
PUVA PLUS TPA
PROMOTION
SKH
HRS
SKH
PSORALEN
PLUS UVA
(PUVA) 36
EXPOSURES
HRS
20
30
40
50
60
70
80
900
1000
TIME AFTER FIRST EXPOSURE (weeks)

ORNL-DWG 94M-2502 BIO



ORNL-DWG 94M-2501 BIO



MULTISTAGE CARCINOGENESIS

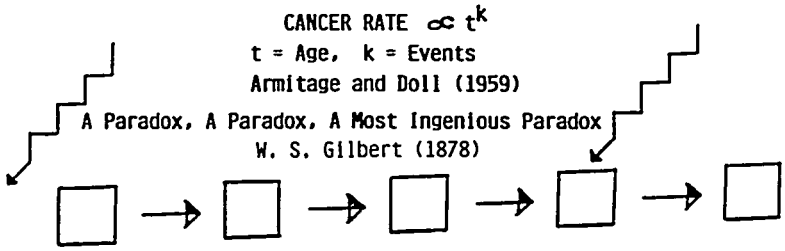
CANCER RATE $\propto t^k$

t = Age, k = Events

Armitage and Doll (1959)

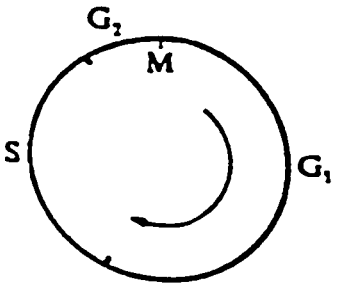
A Paradox, A Paradox, A Most Ingenious Paradox

W. S. Gilbert (1878)

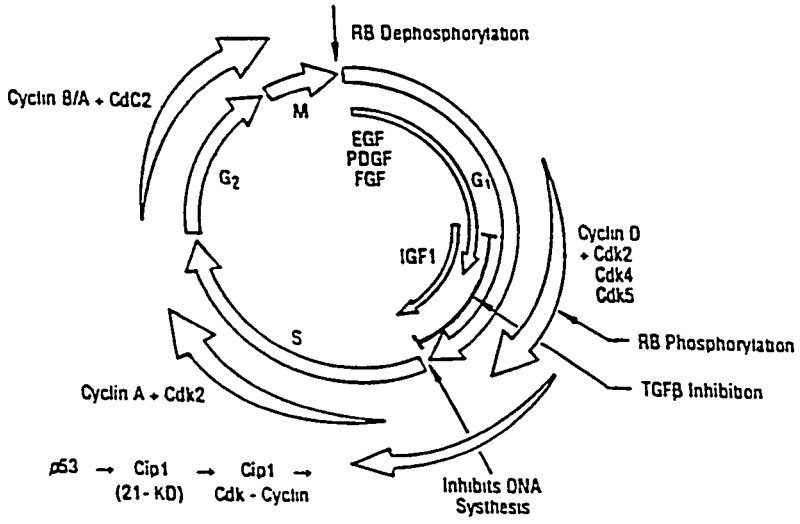


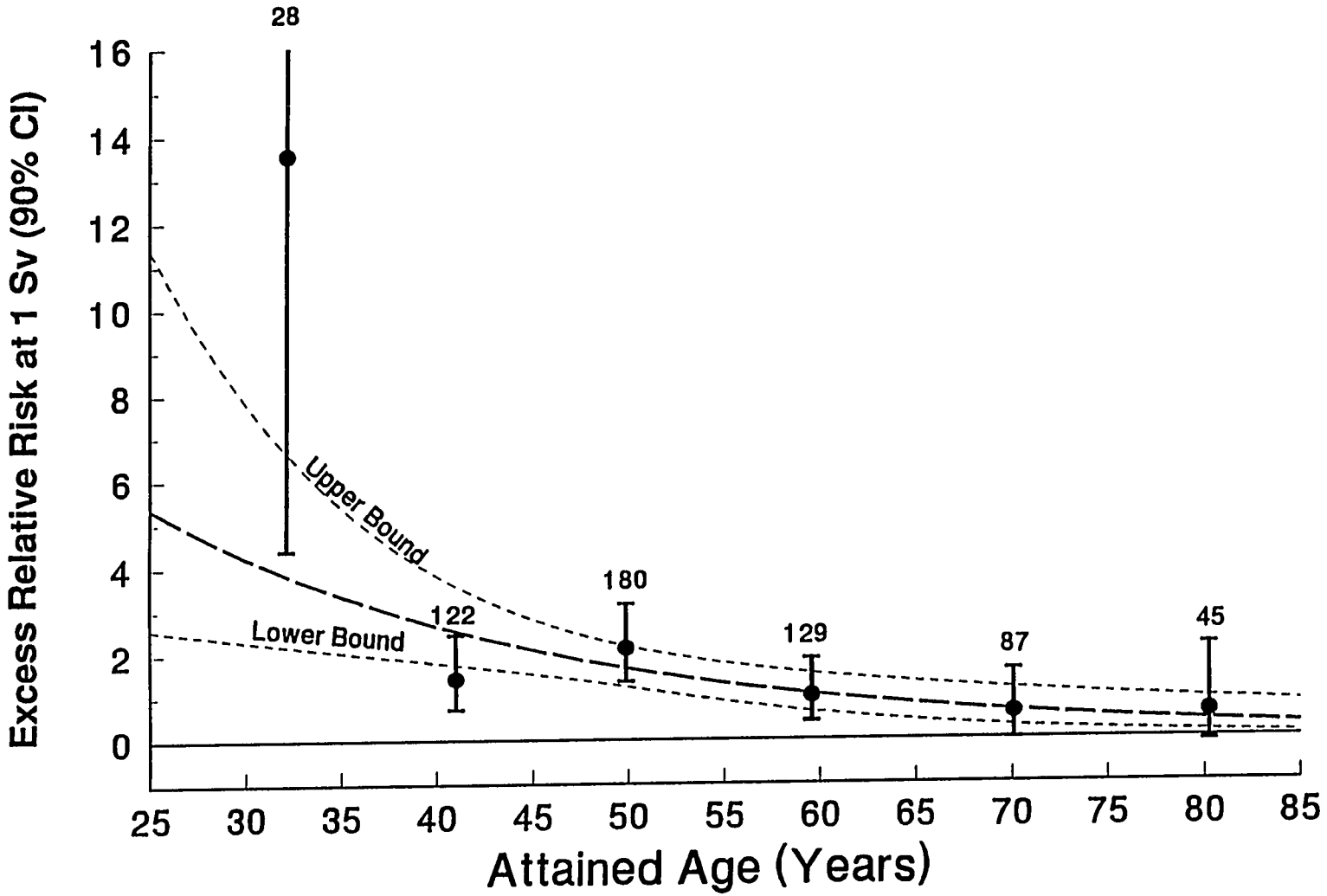
The Cell Cycle

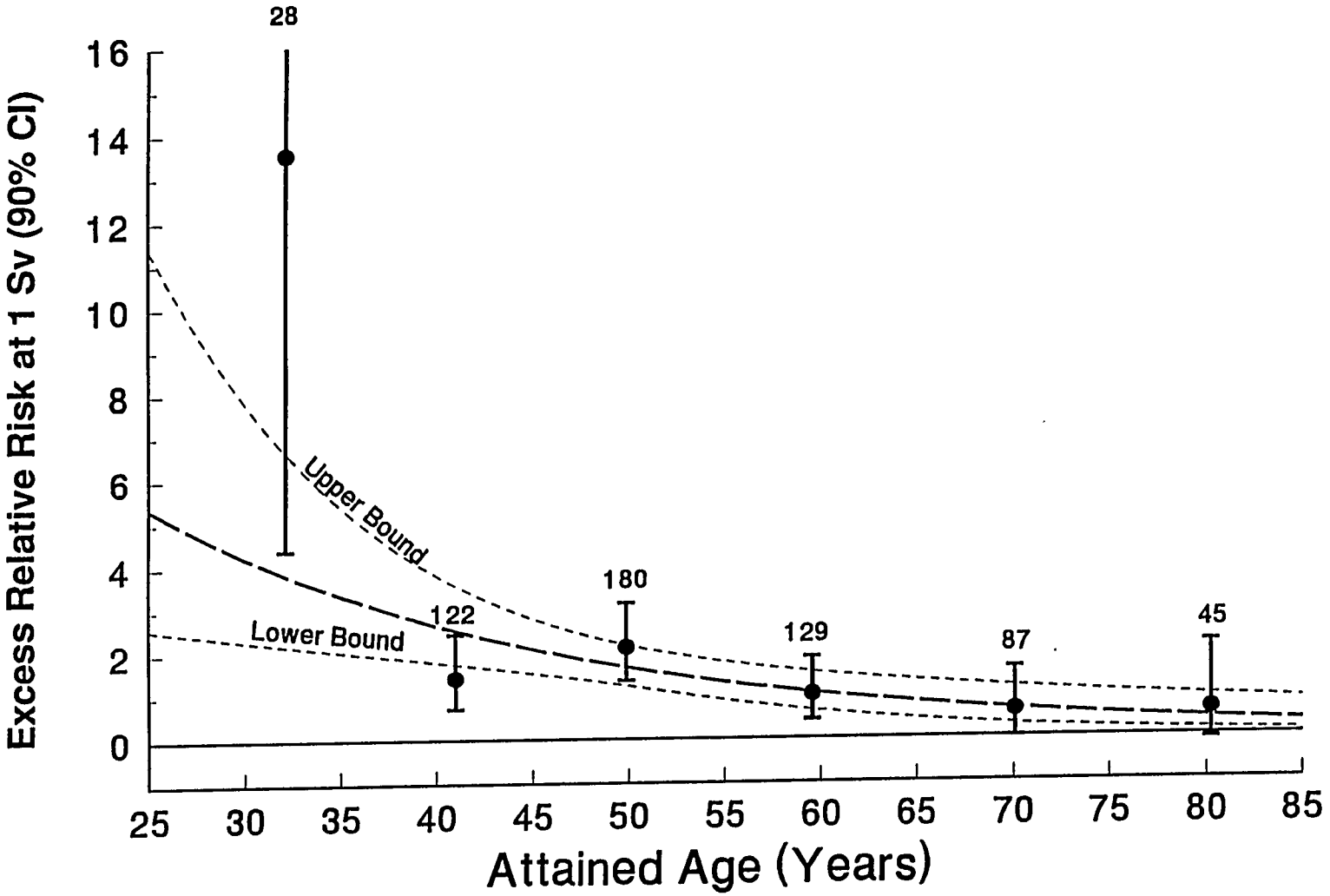
1953



1993




Excess Relative Risk at 1 Sv (90% CI)
16
14
12
10
8
6
4
2
0
28
Upper Bound
Lower Bound
122
180
129
87
45
25
30
35
40
45
50
55
60
65
70
75
80
85
Attained Age (Years)


Excess Relative Risk at 1 Sv (90% CI)
16
14
12
10
8
6
4
2
0
28
122
180
129
87
45
Upper Bound
Lower Bound
25
30
35
40
45
50
55
60
65
70
75
80
85
Attained Age (Years)