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Biology Division Progress Report

For Period of October 1, 1978–May 31, 1980

MASTER

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FOR THE UNITED STATES
DEPARTMENT OF ENERGY

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BIOLOGY DIVISION
PROGRESS REPORT

For Period of October 1, 1978 - May 31, 1980

Date Published: October 1980

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T. T. Odell, Associate Director

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J. L. Epler, Cellular and Comparative Mutagenesis
L. B. Russell, Mammalian Genetics and Teratology
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Introduction

A principal goal of the Biology Division continues to be to increase man's understanding of the effects of environmental agents on the health of human beings. Our strategy has been to use sub-human models (subcellular test tube systems to whole mammals) to assess recognizable effects, such as toxicity, mutagenicity, carcinogenicity and teratogenicity of chemical, physical and biological agents on living systems, and concomitantly to investigate the fundamental mechanisms that are responsible for observed effects. Only by pursuit of a spectrum of studies from the most fundamental to the most applied can our institution maintain a strong scientific presence and provide the information ultimately needed by our human culture to assure a satisfactory co-existence between man's advancing technology and the biologic requirements of living things, including man.

Special emphasis is being placed on problems associated with emerging energy technologies. Thus a wide variety of assays are being developed and utilized to test products and by-products obtained directly from developing energy systems, such as coal gasification and coal liquefaction systems, while at the same time investigations are being carried out to elucidate the biologic mechanisms that underlie the assays and that are the basis for the effects produced in biologic systems by agents released into the environment.

The body of this report provides summaries of the aims, scope and progress of the research of groups of investigators in the Division during the period of October 1, 1978, through May 31, 1980. At the end of each summary is a list of publications covering the same period (published or accepted for publication). For convenience, the summaries are assembled under Sections in accordance with the current organizational structure of the Biology Division; each Section begins with an overview. It will be apparent, however, that currents run throughout the Division and that various programs support and interact with each other.

In addition, this report includes descriptions of two interdivisional projects, an outline of education activities, a listing of the members of the 1980 Advisory Committee, seminar programs in the Division, research conferences sponsored by the Division, extramural activities of staff members, abstracts for technical meetings by staff, and a table that summarizes funding and personnel levels for fiscal year 1980.

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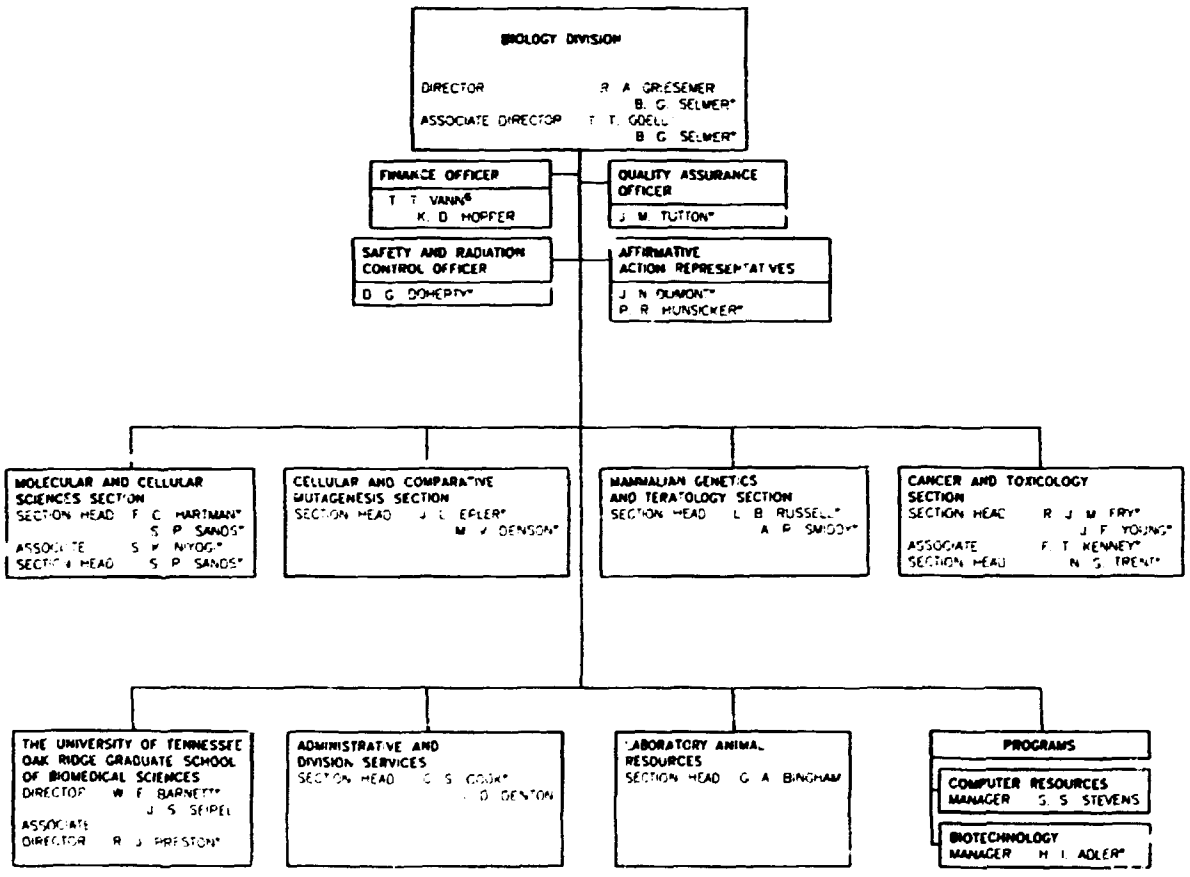

*DUAL CAPACITY
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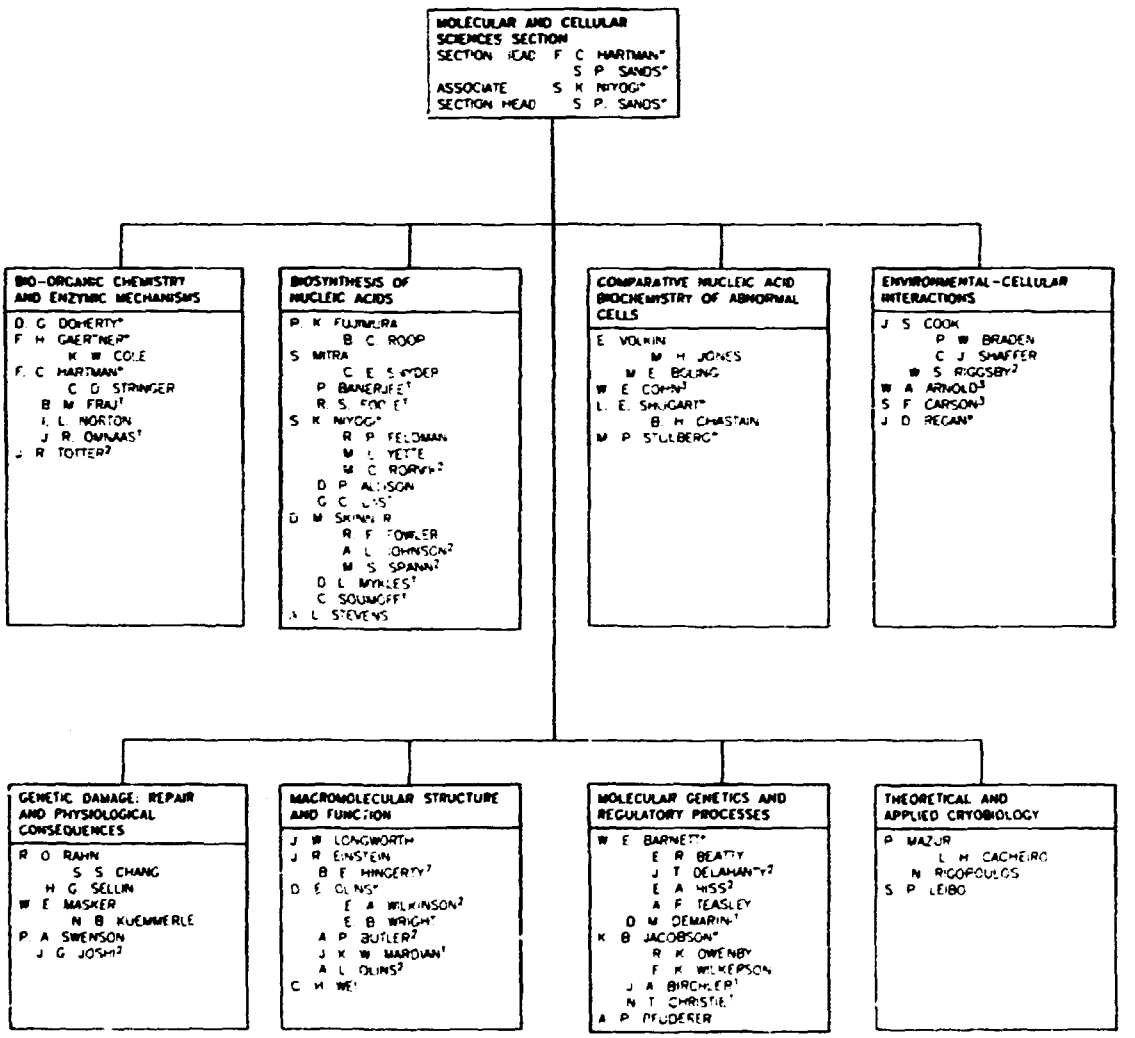
[2]VISITING INVESTIGATOR
[3]CONSULTANT
[4]EUGENE P. WIGNER POSTDOCTORAL FELLOW
[5]LOAN FROM COMPUTER SCIENCES DIVISION
[6]LOAN FROM FINANCE AND MATERIALS DIVISION
[7]LOAN FROM INFORMATION DIVISION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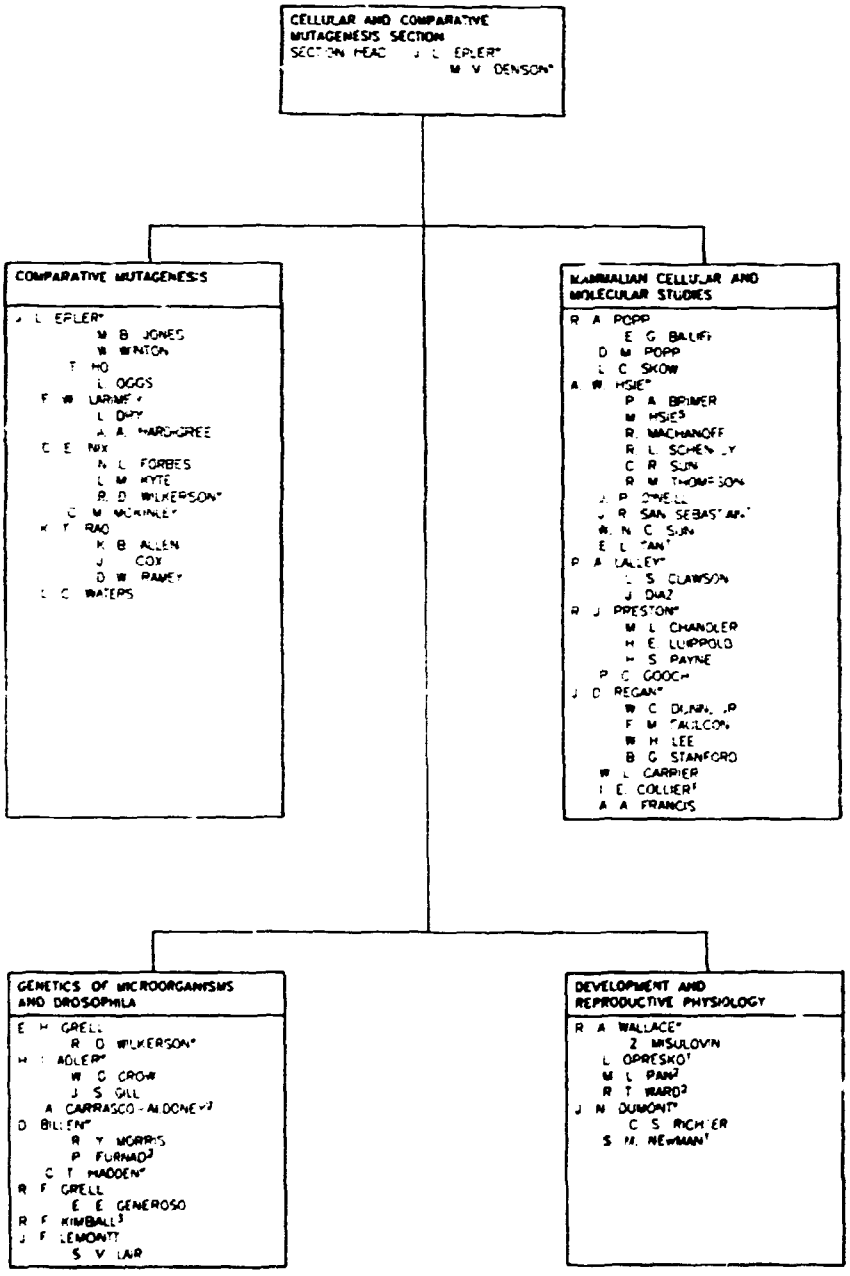
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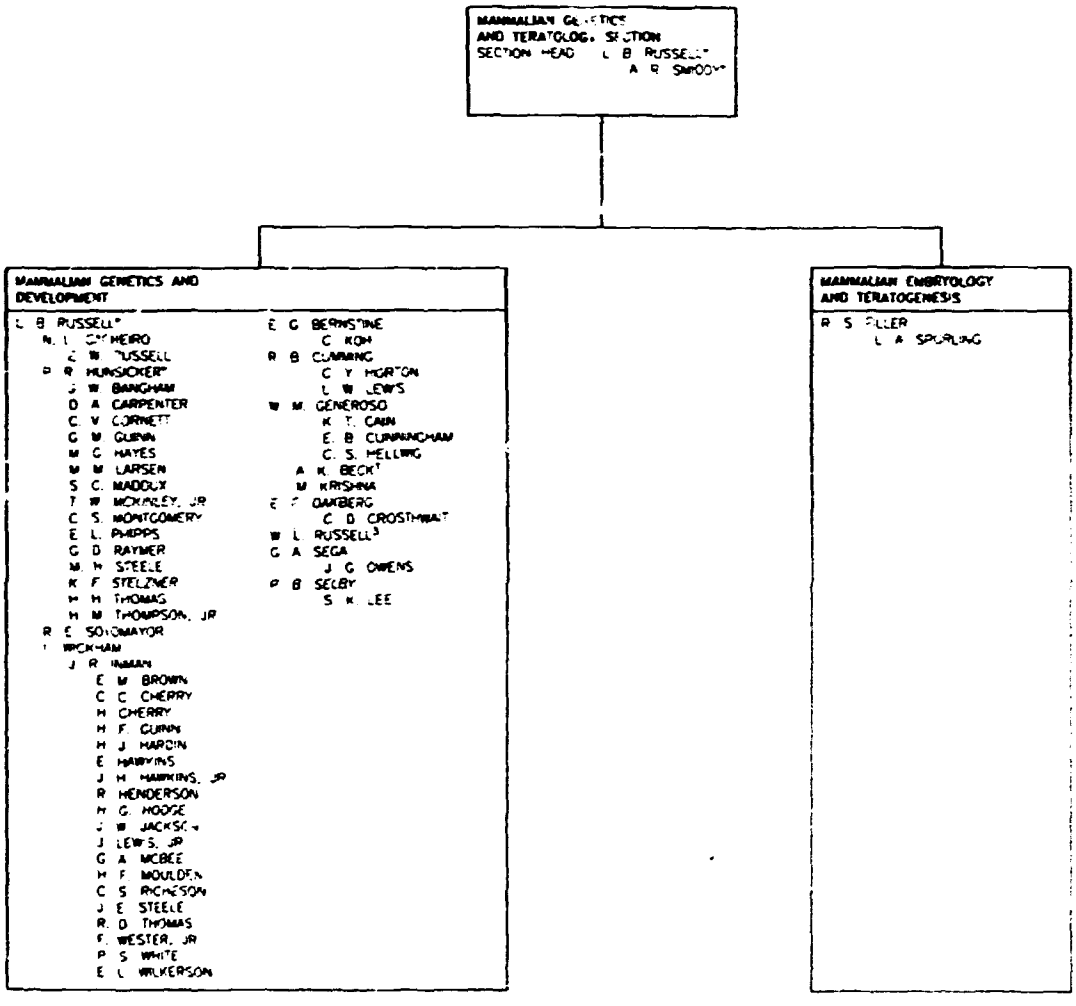
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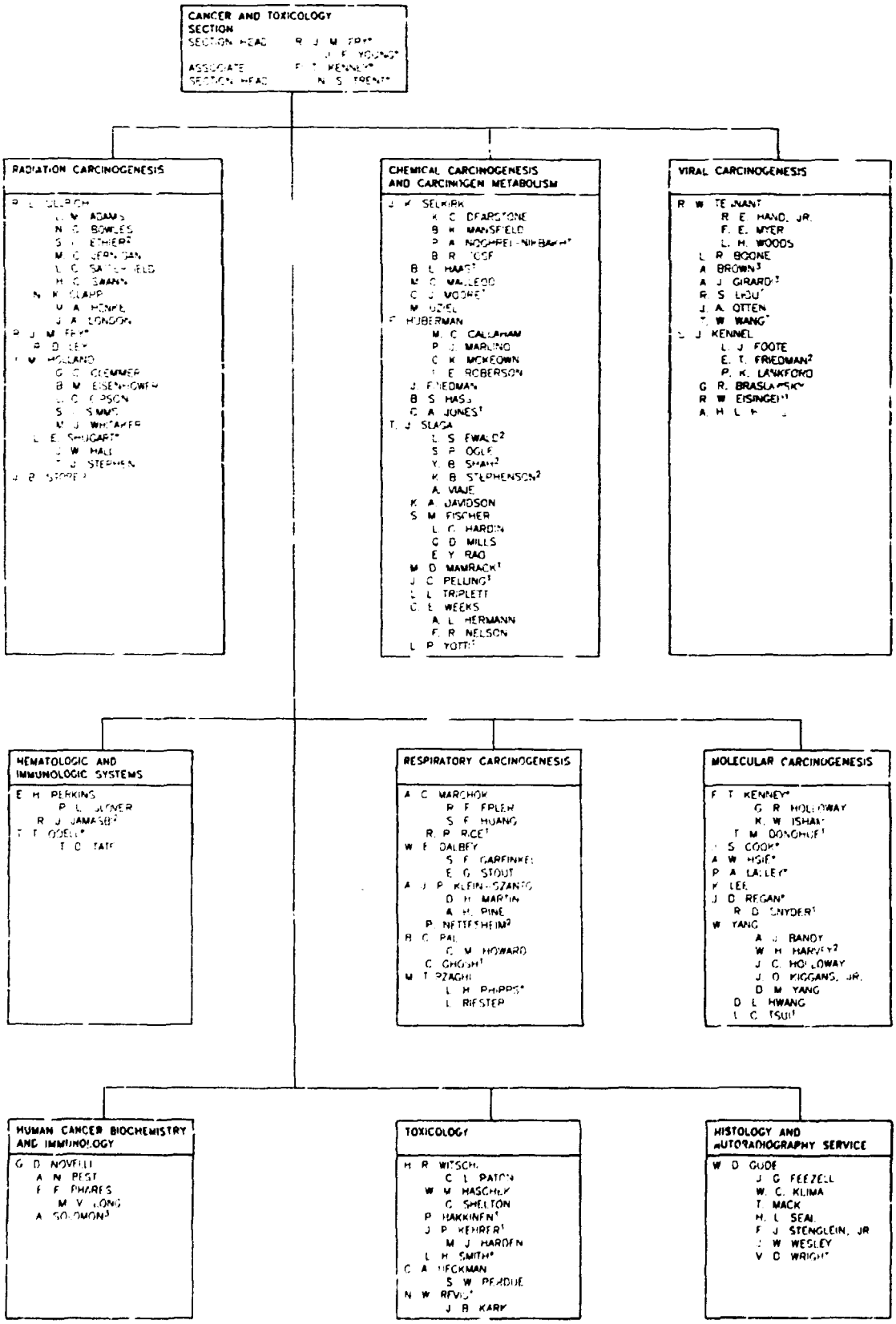
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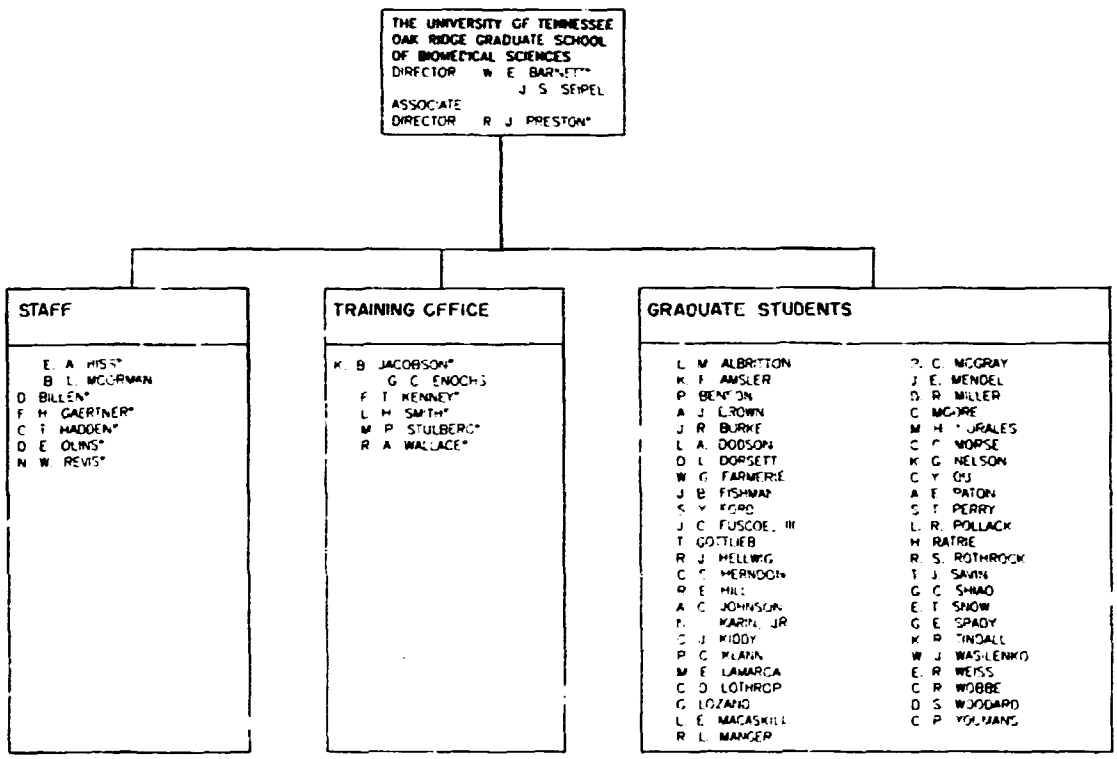
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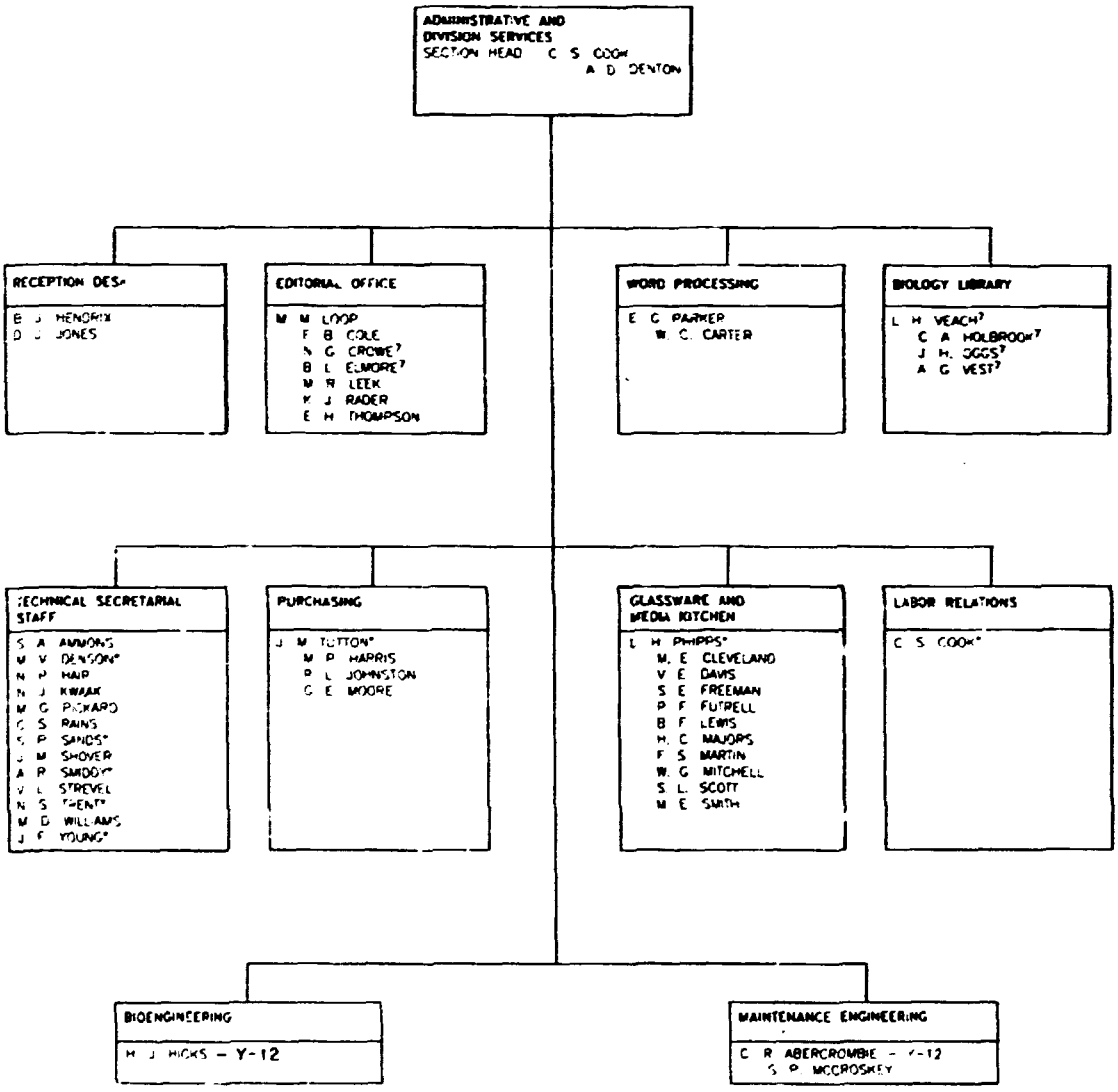
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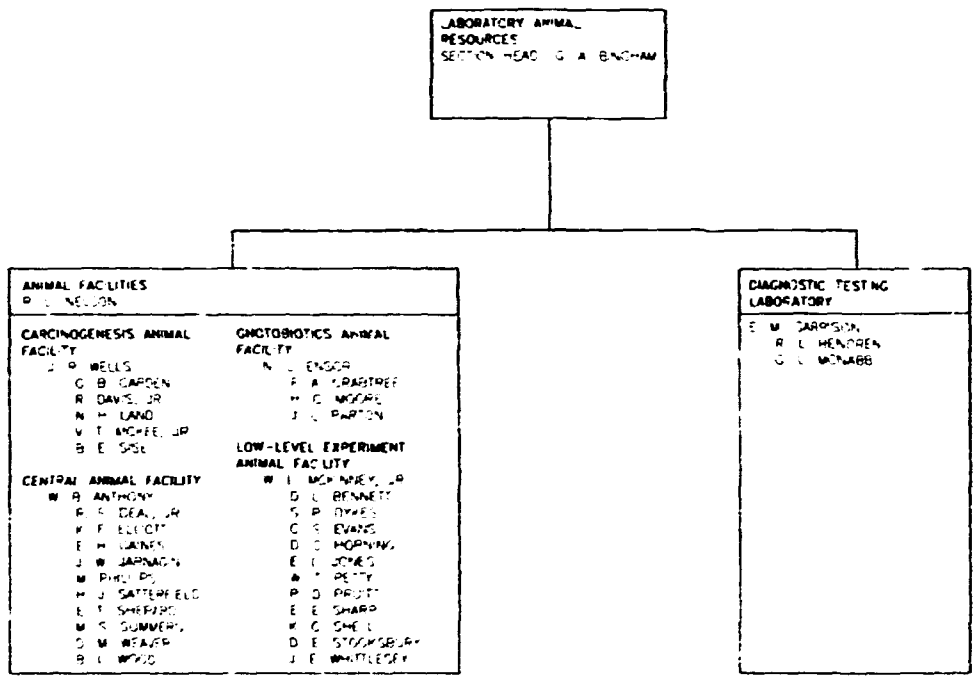
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on Research Summaries

[1]Postdoctoral Investigator

[2]Visiting Investigator

[3]Consultant

[4]Eugene P. Wigner Postdoctoral Fellow

[5]Loan from Computer Sciences Division

[6]Loan from Chemistry Division

[7]Student, University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences

[8]Loan from Health and Safety Research Division

[9]Student, Radiation Biology, University of Tennessee

[10]Student, Department of Microbiology, University of Tennessee

Research Activities

Molecular and Cellular Sciences Sectic .

Section Overview

The costs of energy production and utilization are most commonly calculated in physical and monetary units. However, potentially deleterious effects o. human health and on the environment are equally important elements in the calculations of energy cos's. Such deleterious effects may include cancer, genetic damage, birth defects, and acute toxic poisoning, all of which represent perturbations of exceedingly complex biological processes. Fundamental understanding of these processes is essential if (i) the primary events which lead to clinical manifestation are to be defined; (ii) the mechanisms causing abnormalities of metabolic pathways are to be determined; (iii) prevention and/or amelioration of pathological conditions are to be achieved; and (iv) procedures to estimate risks to humans, animals, and plants from exposure to by-products of energy production are to be designed. The principal aim of the research conducted in the Molecular and Cellular Sciences Section is to obtain such fundamental understanding of diverse biological processes. The premise on which this aim is based is that an understanding of molecular and cellular biology is crucial to the solution of health and environmental problems with which the Department of Energy is charged.

To achieve this aim, a wide variety of scientific disciplines are represented within the Section. One central theme of much of our research is the study of gene structure and function. The reason for this is that the principal consequences of exposure of biological systems to radiation (from nuclear energy production) and to organic and inorganic chemicals (from natural and synthetic fuel production) result from their interactions with the cellular genome. Within this central theme are studies on the structure of DNA and chromatin, the interaction of nucleic acids with heavy metals and certain carcinogenic and mutagenic chemicals, replication and transcription of DNA and their regulation, mechanisms of repair of DNA damaged by radiation or chemicals, and molecular genetics. Some of the Section's major recent contributions in these fields that have also, but not coincidentally, contributed to a more comprehensive understanding of carcinogenesis and mutagenesis include the following:

1. An exoribonuclease has been discovered in Saccharomyces cerevisiae which hydrolyzes rRNA and poly(A) yielding 5'-mononucleotides by a 5'→3' mode of hydrolysis. A specific pyrophosphatase which decaps [^{3}H]methyl capped mRNA of yeast yielding 7 mGDP purifies quite closely with the exoribonuclease and renders capped mRNA susceptible to hydrolysis. The two enzymes may be involved in the in vivo degradation of capped mRNA.
2. The most complex eukaryotic satellite DNA yet studied has been cloned and is being characterized. The satellite is present in 1.6×10^4 copies per genome, but in its 2050 base-pair repeat length there are no simple repeats. It contains both initiator and terminator sequences. Variants on the basic sequences found in the many derived clones permit an estimate of the major evolutionary changes that have arisen in this complex DNA.

3. Nucleosome cores isolated from chromatin have been shown to contain two specific binding sites which can accept either of two high mobility group (HMG) proteins. These proteins, HMG 14 and 17, are known to play a key role in maintaining the unique conformation of transcriptionally competent gene sequences.
4. Parvoviruses are of two types: those that are dependent upon a helper virus for growth and those that are helper-independent. The reported classical distinction between the two subgroups is that helper-dependent parvoviruses contain both (+) and (-) DNA strands whereas the nondefective group contains only (-) DNA strands. Studies with a human parvovirus (LuIII) now show that the so-called "classical distinction" is not universally valid.
5. Plasmid pKM101, when harbored in Salmonella typhimurium, protects the bacterium against the lethal effects of UV and against induced cessation of respiration. A mutant of pKM101 that has no influence against the lethal effect likewise does not influence respiration shutoff. The product of the plasmid UV resistance gene may interact with a respiration shutoff operon.
6. Significant progress has been made toward defining an in vitro replication system for T5 DNA.
7. An insight into one mechanism of mutation suppression in Drosophila has been achieved. The mutant in question produces a gene product (protein) which inhibits sepiapterin synthase, an enzyme involved in the synthesis of a purple eye pigment. The suppressor mutation results in an altered gene product no longer inhibitory to the synthase and thus pigment synthesis is restored.
8. The three-dimensional structure of an adenine-cadmium (II) complex has been determined, thus providing the first known crystal structure of a purine base coordinated to cadmium.
9. From an analysis of the action spectrum for the photolysis of iodine in DNA substituted with either IC or IU, the extent of singlet energy transfer in DNA at 25°C has been shown to occur at a distance corresponding to a nearest neighbor interaction and no further.

Other disciplines within the Section are cryobiology, developmental biology, enzymology, membrane biology, protein biosyntnesis, protein chemistry, and X-ray diffraction. Because of their diversity, research in these areas cannot be so easily categorized as having a central theme. However, the relationships of these activities to the Division's general mission to study adverse health and environmental effects of energy production are described in the group reports that follow. Notable recent achievements in these disciplines include:

1. By use of [^{13}C]amino acid incorporation, a procedure has been devised to measure the turnover of an essential surface-membrane enzyme, Na,K-ATPase. Cells treated with Na,K-ATPase-inhibiting cardiac glycosides, or starved for extracellular K^+, are induced to maintain up to a three-fold enhanced steady-state level of the enzyme. This induction which is specific, does not involve any measurable change in the rate of synthesis of the enzyme but appears to be regulated entirely by a decreased rate constant for removal (degradation) of the ATPase from the cell surface.
2. It has been demonstrated that five of the enzymes catalyzing the biosynthesis of aromatic amino acids in Neurospora crassa are synthesized as a single polypeptide chain that is subsequently assembled into a multidomained pentafunctional homodimer. This enzyme system is readily modified by limited proteolysis via a set of endogenous proteolytic activities.

3. The catalytic site of ribulosebisphosphate carboxylase/oxygenase has been partially characterized. This plant enzyme is essential to the photosynthetic conversion of atmospheric CO_2 to carbohydrate.
4. Two groups of neurotoxins have been identified in shale oil and synthoil. One group is a series of ether soluble bases which are anticholinesterases. The other group consists of phenols, the most common pollutants in synthetic oils. These characterizations have led to the development of screening systems for adrenergic and cholinergic inhibitors in coal tar effluents.
5. It has been demonstrated that slow freezing injury to cells is more strongly correlated with the fraction of solution that freezes than with the composition of this partially frozen solution. This refutes the assumption as to the cause of injury that has been almost universally accepted until now. To calculate the response of cells during freezing requires knowledge of their osmotic water permeability, L_p, and its activation energy, ΔH^*. These values have now been measured for fertilized and unfertilized mouse ova: L_p at 20°C is ~0.4 $\mu m^3/\mu m^2$-min-atm and ΔH^* is ~14 Kcal/mol, values that are comparable to those of other mammalian cells such as leukocytes.

Nucleic Acids: Structure, Function and Interaction with Carcinogens/Mutagens

REPLICATION OF LINEAR DUPLEX DNA IN VITRO USING BACTERIOPHAGE T5 DNA POLYMERASE

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Our goals are to understand processes involved in accurate replication of DNA using linear DNA-phage T5 DNA polymerase systems as a model. We hope to control and modify such processes *in vitro*. These studies may lead to development of specific technology for studies of mutagenic agents on replication processes. Many mutagenic agents are by-products of energy production.

T5 DNA polymerase is essential for replication of T5 DNA. When the structural gene for the T5 DNA polymerase is defective, there is no synthesis of T5 DNA even though three DNA polymerases of the host, *Escherichia coli* are present.

In the past few years we have extensively characterized this polymerase, which was purified to apparent homogeneity, and found it to have several properties distinctly different from other DNA polymerases shown to be essential for replication. The purified polymerase itself is capable of extensive strand displacement as it replicates nicked circular DNA. It is the most processive DNA polymerase we have studied. It binds preferentially to primer ends of primer templates. Like most other prokaryotic polymerases, it has 3'→5' exonuclease associated with it which may play a role in editing of nucleotides being incorporated. This 3'→5' exonuclease was found to be quasi-processive. Thus we have found that a single enzyme bound to a primer end of a primer-template can carry out extensive replication, and its associated exonuclease is capable of editing function by reversing its direction of translocation.

According to the currently most popular model, DNA replication of one of the strands initiates from a unique site (an origin), and replication of another strand is discontinuous,

initiating at multiple sites with no obvious specificity. Initiation processes at both of these strands require primers that are made up of either ribonucleotides or deoxyribonucleotides with free 3'-OH ends. We decided to concentrate our efforts on initiation process at the origin because it is not well understood except in small circular DNAs. In the case of T5 DNA, studies are complicated because preliminary studies suggest that the DNA has multiple origins, and there is a discrepancy between physical entity of the DNA and the genetic map. Physical studies showed that T5 DNA is one molecule, but genetic analysis suggests that T5 DNA is made up of four segments. Attempts are being made to construct a segment of T5 DNA with one origin of replication and also to get better correlation between physical and genetic map of DNA, at least within the region that codes for proteins essential for DNA replication.

Meanwhile, attempts are also being made to establish an in vitro system that will specifically replicate T5 DNA. We have established a crude system with some specificity, and essential factors are being fractionated and identified.

There are several possible directions for this project. Further characterization of the polymerase from wild type and mutants will lead to an understanding of parameters that contribute to accurate replication of DNA. Studies at origin of replication will lead to an understanding of factors that contribute to initiation at a specific site. Studies on correlation between physical and genetic map involve site-specific mutagenesis. We hope that all or any one of these will lead to methods for controlled replication and mutagenesis of specific segments of DNA which may be applicable to replication and alteration of genetic elements from any other sources.

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STRUCTURAL ASPECTS OF DNA IN ITS REPLICATION AND REPAIR

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The research efforts in this laboratory are directed toward understanding the mechanisms of DNA replication and of repair of carcinogen-modified DNAs. DNA replication is studied in a model system of single-stranded linear DNA-containing teratogenic parvoviruses. DNA repair in vitro is being studied with a novel DNA substrate synthesized in vitro.

Structure of Single-stranded DNA Genomes of Autonomous Parvoviruses. Mammalian parvoviruses contain single-stranded (ss) DNA genomes of 1.4 to 2.0 megadaltons and are divided in two subgroups. The helper virus-dependent defective viruses, adeno-associated virus (AAV) of human origin, encapsidate both (+) and (-) strands of DNA while the nondefective teratogenic viruses, Kilham Rat Virus (KRV), H-1 virus of rat, bovine parvovirus (BPV), etc., are believed to encapsidate only the (-) strand DNA. The ssDNA of KRV contains terminal hairpins which can be isolated as nuclease-resistant core pieces of 90 and 150 nucleotides corresponding to the 3' and 5' termini of the genome. In contrast to DNAs from the rat viruses KRV and H-1, which contain 5% of the (+) strand, DNA from BPV contains a significant amount (10-20%) of (+) strand, and DNA from the putative human virus LuIII behaves like that of AAV in having both (+) and (-) strands in equal amount. Furthermore, it appears that LuIII DNA contains homology in its terminal sequence similar to that of AAV, in contrast to the absence of such homology in rodent viruses. Thus the strand specificity of the viral genome is not related to the defectiveness of viruses as earlier believed but may be a reflection of the natural host-type of the virus.

Size Determination of ssDNA in CH_3HgOH-Agarose. The size determination of ssDNA by gel electrophoresis is affected by its secondary structure. We have adapted the procedure of Bailey and Davidson for accurate sizing of ssDNA in the 3 to 10 kilobase range by electrophoresis under denaturing conditions in agarose containing CH_3HgOH. DNAs of rodent viruses (KRV, H-1) contain about 5000 nucleotides, while BPV and LuIII DNAs have 5300-5500 nucleotides.

The Nonidentity of Two Independently Isolated Strains of KRV. Cytotropism of parvoviruses has been well documented. The strains 171 and 308 of KRV cross react antigenically, but we have shown that KRV171 selectively infects normal rat kidney cells and not rat nephroma cells, while KRV308 infects both types of cells. The capsid proteins of the two strains are of identical sizes; however their isoelectric points are slightly different, suggesting a difference in their amino acid sequence. Restriction fragment analysis of viral replicative form (RF) DNAs synthesized *in vitro* shows that the two DNAs have identical restriction cleavage sites for eight endonucleases while one site is different for the two DNAs in the cases of three other restriction endonucleases. These distinct sites appear to be located in 0.6 to 0.8 of the viral genome from the 3' end and may account for the possible differences in the amino acid sequence of the capsid proteins. Although the two viral DNAs are of identical size, KRV308 DNA has a slightly higher electrophoretic mobility in the native state, and this may be a reflection of its different secondary structure.

Replication of KRV DNA in vitro. Because of the small size of parvovirus genomes, their DNA replication is expected to be nearly totally dependent on host components. Purified rat liver DNA polymerases α and β cannot replicate KRV DNA to any significant extent *in vitro*. DNA polymerase I of *Escherichia coli* and T4 and T5 phage DNA polymerases replicate KRV DNA from its 3' hairpin primer to produce double-stranded RF. RF synthesized by T4 DNA polymerase is slightly smaller than that synthesized by T5 DNA polymerase or by *E. coli* DNA polymerase I (Klenow fragment). This suggests, based on the known properties of the enzymes, that the 5' hairpin end of KRV DNA is extended by the latter two enzymes but not by the T4 DNA polymerase.

Mechanism of Repair of DNA Containing O^6-Methylguanine. Nitrosamines cause alkylation of DNA both *in vivo* and *in vitro* at several sites among which O^6-alkylation of guanine is believed to be a carcinogenic, as well as a mutagenic, lesion. The persistence of O^6-alkylguanine can be related to tissue-specific tumorigenesis. The mechanism of removal of

O^6-alkylguanine from DNA is not known although recent experiments of Lindahl and Cairns suggest that an inducible protein in E. coli removes the alkyl group from the modified base in a stoichiometric fashion. We prepared a synthetic substrate of poly dG:poly dC in which G is occasionally substituted by [8-^{3}H]O^6-methylG. We were able to use this substrate to confirm the experiments of Karran et al. (Nature 280, 76, 1979) that an inducible activity that is undetectable in uninduced E. coli is able to demethylate O^6-methyl G in DNA. Our result provides the direct evidence that demethylation of the alkylated base occurs at the DNA level. Experiments with uninduced rat liver extracts showed that, contrary to earlier observations, demethylation did not occur at the DNA level. Further experiments are in progress to investigate if induction of a demethylase activity does occur in rat liver and to exclude other possible mechanisms of removal of O^6-alkylG.

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MECHANISMS OF DNA DAMAGE AND REPAIR

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DNA damage can result in several detrimental effects with consequences to living organisms that can be fully appreciated only by understanding the cell's response to genetic lesions on a molecular level. Our research is directed toward studies of the molecular mechanism by which damaged DNA is repaired and the effects of damage on DNA metabolism. Some of these studies use cell-free systems capable of (i) replicating bacteriophage T7 DNA, (ii) performing steps of excision repair, (iii) carrying out genetic recombination in vitro, and (iv) maturing and encapsulating T7 DNA. This system can produce viable phage in vitro with good efficiency by replicating T7 DNA and then packaging the product into phage heads. Our recent studies with this system have emphasized the interconnection between DNA replication and recombination and have provided details on the in vitro response of both these DNA metabolic processes to damage inflicted by ultraviolet radiation. This work is now being extended to include studies performed with potentially mutagenic chemicals. Additionally, our experiments have yielded information regarding the enzymes and types of DNA structures involved in recombination, maturation, and packaging of T7 DNA. This work is complemented by in vivo studies using mutants of Escherichia coli deficient in enzymes thought to be important in DNA repair, including the product of the uvrD gene, a protein involved in mutagenesis as well as DNA repair. This work has contributed information on the variety of biochemical pathways employed during the repair process.

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STRUCTURE AND FUNCTION OF THE GENOME OF SIMIAN VIRUS 40

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Studies of the control of transcription and replication, particularly the specific initiation of synthesis, are essential for understanding molecular mechanisms of viral carcinogenesis. In higher eukaryotes, transcription and replication occur at the level of chromatin, that is, DNA associated with histones, acidic proteins, and other regulatory proteins. Therefore, studies of the structure and function of eukaryotic chromatin are crucial for elucidation of gene duplication and expression.

Simian virus (SV)40 provides an ideal model for such studies. It belongs to the class of papova viruses, which are lytic and tumorigenic DNA viruses. The SV40 genome is a supercoiled circular DNA, M_r 3.6 x 10^6, and contains three coat proteins and the histones H2A, H2B, H3, H4, and also H1, and nonhistone proteins in its functional state.

We find that SV40 minichromosomes from virions (MV) and infected cells (MI) have highly compact structures in buffer containing 0.15 M NaCl and sediment with S values of about 90-100 and 115-130, respectively. Under the electron microscope, MI appear to be the more compact of the two. Only 30-35% of the sites of origin and termination of replication in MV are freely available to the restriction endonucleases Bgl I and Bam HI. MV are similarly resistant to Eco RI and Hpa II. In contrast, almost no sites in MI are available to any of the above single-cut endonucleases. In 0.6 M NaCl, MV and MI change to relaxed structures of 45-55 S and 50-60 S, respectively, containing 20-24 nucleosomes per genome, and become more sensitive to Bgl I, Bam HI, Eco RI, and Hpa II. Our results show that the states of chromatin in mature virions and inside the nuclei before encapsidation are not identical. The nonavailability of the sites to the restriction endonucleases in the MI suggests that the distribution of nucleosomes in the compact form may not be random. One might obtain results supporting random distribution of the nucleosomes only when the superstructure is destroyed and nucleosomes are separated by DNA spacers, along which the movement of the nucleosomes is then possible. Work is now in progress to analyze the state of the minichromosome inside the mature virions and in the cell nuclei and their possible interactions with different enzymes and proteins.

It had previously been shown that the minichromosome isolated from infected cells contains host RNA polymerase II bound in a viral transcription complex, while that from mature virions does not contain the enzyme. In a preliminary study of this endogenous RNA polymerase activity, we find that the most compact form of the minichromosome still has the bound

polymerase but the transcriptional activity (as tested by added ribonucleoside triphosphates) is low, probably due to restricted movement of the bound polymerase. As the minichromosome unfolds at higher salt concentrations, the transcriptional activity increases, suggesting freedom of movement for the polymerase. Increasing salt concentration also results in the conversion of some of the polymerase from its bound form to a "free" form, as measured by the synthesis of poly(rA-rU) upon addition of poly(dA-dT) template and labeled ATP plus UTP.

We have found that nuclear, but not cytoplasmic, extracts of the host BSC-1 cells can assemble nucleosome-like structures at physiological ionic strength with SV40 DNA and the four core histones H2A, H2B, H3 and H4 from either homologous or heterologous systems. The nature of the assembly activity is under investigation.

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CHROMOSOME CHEMISTRY

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Our laboratory is devoted to investigation of the structure of nuclei and chromosomes, and two major classes of techniques are employed – biophysical (spectroscopic, hydrodynamic, electrophoretic) and electron microscopic. The current goal of the biophysical part of the laboratory is to determine the structure and conformational states of mononucleosomes. In conjunction with these techniques, we are pursuing a crystallography project aimed at obtaining high resolution data on the detailed structure of core nucleosomes. Presently the electron microscopy is directed towards the localization of specific nuclear macromolecules in the three-dimensional space of nuclei and chromosomes, employing stereo-electron microscopy (stereo-EM) and immunoferritin labeling.

Most of our recent studies have been concerned with the structure and conformational states of the nucleosome as induced by both solvents and individual nonhistone chromosomal proteins belonging to the high mobility group (HMG) (1-4, 7, 8, 12-15). We have found that nucleosomes contain two specific binding sites for HMG-14 and 17. These proteins are believed to be instrumental in maintaining the unique conformation of those portions of chromatin which are capable of being transcribed. Some of our effort is also directed towards elucidating the arrangement of nucleosomes in the higher-order structures of interphase and mitotic cells (10). One of the most useful techniques we are using for these studies is that of stereo-EM (9-11). Using this method, we have examined thin sections of isolated chicken

erythrocyte nuclei at various tilt angles. We have observed a parallel alignment of 25 nm chromatin fibers adjacent to the nuclear envelope and have demonstrated a fiber structure consistent with close-packed arrays of nucleosomes.

It is probable that these higher-order structures change during DNA replication, mitosis, and RNA transcription. In fact, it is likely that the conformation of the nucleosome responds to alterations in chromosome physiology. Besides furnishing essential information toward understanding the mechanisms of gene expression, these studies are fundamental to interpreting preferred sites of carcinogen binding (5, 6) and the availability of different regions of the DNA to repair processes.

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THE STRUCTURE AND ORGANIZATION OF THE EUKARYOTIC GENOME WITH SPECIAL EMPHASIS ON VERY HIGHLY REPEATED SATELLITE DNA

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All of the information for the activities of all cells ultimately resides in their DNAs. Specific portions, from 1 – 30%, of the DNAs of most eukaryotes are comprised of very highly repeated sequences. Although these DNAs were originally thought to be molecules of very simple sequence, we and others have isolated and characterized several with substantial complexities. Some of these specific sequences of eukaryotic genomes are isolable as distinct entities by centrifugation in CsCl or Cs_2SO_4 density gradients. We refer to them as "patent" satellites. Others must be separated by binding to ligands, or by the rapidity with which they reassociate after dissociation, or by treatment of total DNA with restriction enzymes. We refer to these as "cryptic" satellites since they are otherwise indistinguishable from the major component DNA.

We study satellite DNAs in Crustacea because in these organisms they are frequently found as a very significant component of the total, and because we have developed methods for controlling the growth cycle of these organisms and can thus relate nucleic acid metabolism to growth. Our studies on satellite DNAs include analyses by reassociation kinetics, restriction enzyme mapping, and determination of the primary sequences following base-specific chemical degradations. Under intensive study at present are a pair of cryptic satellites that comprise 30% of the total DNA of one species, a hermit crab, and a G+C-rich patent satellite with a repeat length of 2.05 kilobase pairs (kb) which comprises 3% of the total DNA of another species, a Bermuda land crab. The former has at least two populations of sequences. Both sequences contain runs of A and T residues interspersed with other sequences rich in G and C residues. In one, with a basic repeating unit of 80 bp, the runs of A's alternate with runs of T's. In the other, with a basic repeating unit of 170 bp, the number of runs of A's interrupted with G+C-rich sequences increases as the number of runs of T's similarly interrupted by G+C-rich sequences decreases along the basic repeat unit. We also observe multimers of both of these types of sequences caused by the loss of one or more restriction sites.

The G+C-rich satellite of the Bermuda land crab, by direct sequence analysis, contains no simple repeated sequence, although some sequences from 17 – 300 nucleotides long may be repeated 2 – 25 times. Both initiator and terminator sequences are seen, similar to 5S DNA of various organisms. This satellite has been cloned. From observations on the clones as well as from our previous work on restriction enzyme patterns and the sequences of the cellular (as opposed to cloned) satellite, it is clear that there are numerous variants. The variants isolated and amplified in the 150 clones on hand should permit us to estimate the major evolutionary changes in this, the most complex satellite yet described.

Our parallel studies on the regulation of the Crustacean molt cycle are geared both to the understanding of this cycle in its own right as well as to our long range goal of relating satellite DNA function to growth. We continue work on both the positive and negative hormonal controls of molting as well as on the phenomenology of the cycle. The phenomenon that has been under most recent investigation has been the physiologically normal degenera-

tion and reformation of somatic muscle necessary to successful ecdysis. We find the specific loss of thin (actin) filaments during the late premolt period when the animal is preparing to shed its old exoskeleton and grow. The factors regulating this highly specific protein degradation are under investigation.

Since we can synchronize populations and obtain RNAs from animals at each stage of the molt cycle, we hope to catch transcripts of a complex satellite whose transcription is restricted to specific developmental stages. Finally, even if transcripts cannot be demonstrated, the information on the primary sequences of specific satellite DNAs will be of predictive value in unraveling the function of these diverse and ubiquitous molecules.

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THE MECHANISM AND CONTROL OF mRNA DEGRADATION

Audrey Stevens

The turnover of mRNA is a fundamental process in molecular biology. Each mRNA turns over at a distinct rate and that rate determines part of its efficiency as a messenger. Control of the level of certain proteins may be found at the level of degradation of mRNA. In order to understand this process, enzymes involved in the degradation of mRNA in Saccharomyces cerevisiae are being studied. An exoribonuclease has been purified 1000-fold from the ribosome fraction. The enzyme is unique in that it hydrolyzes RNA in a 5'→3' direction with the production of 5' mononucleotides. The enzyme hydrolyzes both poly(A) and rRNA in a processive manner. Gel electrophoresis of the enzyme suggests a M_r of about 165,000. The enzyme fraction hydrolyzes (methyl-^{3}H-labeled) 5'-capped mRNA from yeast at 5-10% of the rate of rRNA. Investigations of this hydrolysis suggest that there is also decapping activity (pyrophosphatase) in the highly purified enzyme and that this activity purifies quite closely with the exoribonuclease activity. Current studies are concentrated on the mechanism of the pyrophosphatase reaction and on further purification of the degradative complex. The results suggest that the two activities may be the 5' capped mRNA degradative enzymes. Hydrolysis of mRNA would then follow the same direction (5'→3') as that of transcription and translation, and no incomplete polypeptide chains would result from interrupted translation. Studies on factors that may influence the activities of the enzyme fraction and be involved in the control of mRNA degradation will be carried out.

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NUCLEIC ACID BIOCHEMISTRY OF CELLS

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The in vivo synthesis of RNA and DNA is dependent to a great extent on the efficiency of the enzymic reactions in the biosynthetic pathways that produce the nucleoside triphosphate precursors. Most of the reactions leading to the production of those macromolecules are highly interrelated and subject to a variety of feedback mechanisms. We have recently found, using a number of different cell lines in culture, that inhibitors of protein synthesis (i.e., cycloheximide, other glutarimides, and puromycin) not only block DNA synthesis and largely inhibit RNA synthesis but also acutely suppress the formation of cytidine and guanosine triphosphates (CTP and GTP). The inhibition by these drugs is effective in both the salvage and de novo synthesis pathways for both pyrimidine and purine compounds. As a result, there is a severe reduction in the availability of newly synthesized precursors for RNA synthesis and their deoxy derivatives for DNA synthesis. At the same time, however, the pool sizes of these precursors, as well as those of uridine and adenosine triphosphate (UTP and ATP), actually increase during this period of inhibition. We have found that the source of these compounds in the pool is from recycling of products produced by degradation of about 15% of preformed nucleic acids. Thus there is, in fact, not a reduction of total nucleoside triphosphate precursors but only of newly synthesized material. These results raise a number of questions that we are currently investigating.

Does the accumulation of acid-soluble products from nucleic acid degradation lead to blocked interconversions of the nucleotides by some sort of feedback inhibition Although this remains a possibility, certain observations deem it unlikely. Are the primary sites of inhibition of DNA and RNA (mostly rRNA) synthesis associated with the polymerase reactions, or are these inhibitions of macromolecular synthesis a secondary result of inhibition in nucleotide production, thereby resulting in a depletion of requisite newly synthesized critical precursors? In this regard, does compartmentation of functional versus relatively nonfunctional nucleotide pools for nucleic acid synthesis play an important role? Is the action of these protein synthesis inhibitors directly on the enzymes presumed to be effected (e.g., CTP synthetase, GMP synthetase) or the indirect consequence of some other drug-mediated alteration of metabolism? Recently, we have found still another perturbation in the nucleotide pathways caused by protein synthesis inhibitors. This is the inhibition of the conversion of deoxyuridylate to thymidylate for DNA synthesis. Is thymidylate synthetase or a deoxyuridine kinase reaction affected by these inhibitors?

Finally, these data suggest the possibility that, in the intact cell, DNA polymerase(s), RNA polymerase(s), and a large number of the enzymes necessary for the formation of the necessary proximal nucleoside triphosphate precursors, as well as some regulatory short-lived protein(s), may all be part of one multienzyme-complex structure.

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ENZYME AND GENE REGULATION IN DROSOPHILA

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Mutations occur as a result of damage to DNA that is caused by chemicals and radiation. Our aim is to understand how mutations are expressed and how this expression can be modified or completely reversed by natural processes.

The scope of these studies involves the examination of Drosophila melanogaster by both biochemical and genetic means. A major question is how the suppressor mutant $su(s)^2$ restores a normal phenotype to flies carrying the mutations vermilion (v), purple (pr), speck (sp), and sable (s). Various subsidiary projects have arisen that involve measurement of the changes in gene expression as a function of age, of gene dosage, and position of the gene in the chromosome.

Since October 1978 in the suppressor study we have: (i) isolated new alleles of pr, constructed genetic combinations involving various alleles of su(s) and either v or pr, and obtained a translocation (X;2) that placed $su(s)^+$ on chromosome 2R (Dr. E. H. Grell performed these genetic experiments.); (ii) shown that pr^+ translocated from 2 to Y is not expressed normally and behaves in a variegated fashion; (iii) shown that the expression of pr is inversely proportional to the dose of $su(s)^+$, indicating that the product of $su(s)^+$ rather than $su(s)^2$ must be understood to determine the mechanism of suppression; (iv) devised two new assays for sepiapterin synthase, one using high-pressure liquid chromatography and the other a spectrofluorometer; (v) established that drosopterin biosynthesis can be studied in vitro and that sepiapterin is not a precursor to drosopterins; (vi) shown that an unidentified pteridine discovered earlier in this laboratory is probably a direct precursor of drosopterin and shares a common precursor with sepiapterin (The study of this precursor is also under way.); and (vii) devised an assay for a product of the $su(s)^+$ gene.

In related projects since October 1978 we have (i) examined the manner by which certain Q-containing tRNAs are altered as a function of age, nutrition and genotype; (ii) determined the presence of tRNA-guanine transglycosylase and guanine-accepting tRNAs in Drosophila (These studies are a part of earlier studies on $tRNA^{Tyr}$ and the control of the presence or absence of queuine in the tRNA and are becoming closely involved in a study of the effects of toxic metals on Drosophila that is described in another report.); (iii) extended studies on aging in Drosophila to regulation of isoacceptors of serine tRNAs by gene dosage.

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MOLECULAR GENETICS

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Effect of Mutagens/Carcinogens on the Rate and Fidelity of Transcription. The identification of mutagens/carcinogens in the environment is now an important social concern. However, because of the time, expense, and insensitivity of most in vivo animal carcinogenicity tests, much effort is now being expended to develop and validate short-term in vitro assays that can be used to monitor the genotoxicity of environmental chemicals. In accordance with the somatic mutation theory of cancer, most of the research in this area has properly focused on the ability of chemicals to cause damage to DNA. However, it is conceivable that mutagens/carcinogens may have epigenetic effects. One way in which such phenotypic aberrations may occur is if the flow of information between DNA and RNA and between RNA and protein is disrupted. To date, virtually no experiments have been reported that would implicate mutagens/carcinogens as disruptive to the accuracy of this flow of information. Although there have been several reports on the effect of the in vivo administration of a mutagen (AAF) on transcriptional fidelity and on the effect of metal salts on the rate and initiation of transcription, there have been no rigorous attempts to examine the effects of mutagens on transcription in vitro. Therefore, in an effort to explore some of the possible epigenetic effects of chemical mutagens/carcinogens, we have begun to examine the effects of such chemicals on the rate and fidelity of transcription in a cell-free system.

In order to study the effect of mutagens/carcinogens on the rate of transcription, we add varying concentrations of a particular chemical to a reaction mixture composed of a DNA template [poly (dA·dT)], Escherichia coli RNA polymerase, complementary ribonucleoside triphosphates (ATP and labeled UTP), $MgCl_2$, and buffer. The reaction is run for 30 min at 37°C, and the radioactivity of the acid-precipitable material is determined. In order to determine the effect of mutagens/carcinogens on the fidelity of transcription, varying concentrations of a particular chemical are added to a reaction mixture composed of the poly (dA·dT) template, RNA polymerase, unlabeled UTP and ATP, $MgCl_2$, buffer, and a radioactively labeled incorrect ribonucleoside triphosphate (either CTP or GTP). The radioactivity of the acid-precipitable material is an indicator of the extent of misincorporation into RNA.

We are currently studying the effect of known mutagens such as ethyl methanesulfonate, methyl methanesulfonate, and N-methyl-N'-nitro-N-nitrosoguanidine, and other alkylating agents, cross-linking agents, and carcinogenic hormones in this system. Although the epigenetic effects of these agents have been largely ignored, they may be important in their overall effect on our genetic health.

Nuclear-organellar Interrelationships. There are essentially no higher forms of life that are not totally dependent upon functional mitochondria and/or chloroplasts for survival, petite mutants of yeast being the sole exception of note. Since both organelles have unique and functional genomes of their own, it appears imperative to understand how these organelles and their genetic information act in concert with the nuclear genome to produce an operationally normal eukaryotic phenotype. Thus, we are continuing our experiments using organellar tRNAs as a probe into the genetic origin of organelle genes and their importance in cell maintenance, performance, and continuity. More specifically, our objectives are purification of organellar and cytoplasmic tRNAs to homogeneity and determination of their sequences. It is anticipated that such information will provide insight into such issues as whether or not organellar tRNAs represent nuclear genes that have been duplicated and compartmentalized, or whether these molecules are transcripts of a prokaryotic genome that endosymbiotically invaded prokaryotic cells.

Our experiments take advantage of the fact that the structure (sequence) of tRNAs has been more rigorously conserved during evolution than any other biological micromolecule. For example, the $tRNA^{Phe}$'s (of known sequence) of all animals indicates such diverse creatures as man and salmon (the fish) are identical. There are many other examples, and it is clear that similarities in tRNA sequence are good indicators of phylogenetic relatedness. Our sequence studies have led to the following conclusions: (i) chloroplastic tRNA cistrons almost certainly did not evolve from nuclear tRNA cistrons, (ii) they probably did evolve from a prokaryotic blue-green algae type cell; (iii) Euglena gracilis, generally considered a green algae, is in reality an animal cell which contains chloroplasts; and (iv) the chloroplasts of Euglena evolved from the same organism as did the chloroplasts of higher plants.

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PROTEIN SYNTHESIS: ABERRANT CONTROL

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The purpose of this project is to identify abnormalities in the regulation and control of mammalian protein biosynthesis and nucleic acid modifications manifested by mutagenesis, viral carcinogenesis, and senescence. Approaches utilized are: (i) In vitro protein synthesis systems, depleted for individual components, are used to test components from abnormal tissues for capabilities to support specific protein synthesis with fidelity. If infidelities are detected, the mechanism responsible is determined at the molecular level. (ii) Specific end products of in vivo protein biosynthesis from organisms exposed to conditions promoting abnormalities are examined for fidelity. If alterations are discovered, the mechanism responsible is determined at the molecular level by use of in vitro systems. (iii) Components of mRNA translation in protein synthesis systems are examined for their involvement in regulation and control. (iv) Enzymes catalyzing modification of nucleic acids involved in protein synthesis are examined for their mechanisms of action. The metabolism of the products of these reactions is elucidated to determine their involvement in regulation and control of specific nucleic acid interactions.

Transfer RNAs from heart, kidney, liver, and spleen of mature and aged mice were tested for their ability to translate EMC viral RNA in a tRNA-dependent cell-free system derived from mouse ascites tumor cells. No significant age-related differences in either the efficiency or fidelity of protein synthesis were discovered, indicating that alterations in tRNAs are probably not involved in the cellular aging of these tissues. Drosophila tRNAs containing Q base vary in Q base content depending on age, nutrition, genotype, and stage of development. We tested both deficient and enriched Q-containing tRNA for a possible role in regulating protein synthesis in the tRNA- and mRNA-dependent in vitro translating system. No effect on the rate, extent, or fidelity of protein synthesis was detected between Q-rich and Q-deficient tRNA.

A KCl extract of rabbit reticulocyte ribosomes has been demonstrated to markedly stimulate the translation of various messenger RNAs in a cell-free system from ascites tumor cells. In contrast, the translation of EMC viral RNA is strongly inhibited by the same extract. Fractionation of the KCl extract allows the separation of these inhibitory and stimulatory activities. The inhibitory activity has been shown to be the consequence of an unusual endonuclease, associated with ribosomes, that produces approximately 4 S products from the degradation of globin mRNA and viral RNA.

The three relatively tightly associated tRNAs of AMV viral RNA were tested for their capability of modulating translation of the RNA in a reticulocyte lysate translating system. By stepwise thermal dissociation of the tRNAs, it was demonstrated that none of these tRNAs influenced the rate or the fidelity of translation. Likewise, with the exception of the primer tRNA, none influenced the rate or product formation of reverse transcription.

Structural analogues of adenosylhomocysteine have been tested as inhibitors of a tRNA (uracil-5-)-methyltransferase preparation from Escherichia coli. All analogues tested gave linear competitive inhibition kinetics with adenosylmethionine as the variable substrate. Comparison of the K_i values obtained leads to the following conclusion concerning the specificity of the adenosylmethionine-adenosyl homocysteine binding site on the enzyme: (i) the terminal amino group of the amino acid moiety is necessary for activity; (ii) both a

chiral change of the asymmetric carbon atom of homocysteine and the presence of the terminal carboxyl group contribute little toward inhibitory inactivity; (iii) analogues in which the amino function of the adenyl moiety are modified or substituted are still potent inhibitors; (iv) inhibitor specificity is considerably reduced when adenine is replaced by a pyrimidine base.

An enzyme activity that brings about the phosphorolysis of methylthioadenosine has been isolated from Drosophila melanogaster and purified approximately 100-fold. The enzyme activity, which has an absolute requirement for phosphate ion, cleaves methylthioadenosine and produces equivalent amounts of adenine and methylthioribose-1-PO_4. The enzyme catalyzes the reverse reaction and has been studied kinetically. Although initial velocity measurements indicate a Ping-Pong mechanism, isotopic exchange studies did not substantiate a methylthioribosylated enzyme intermediate. The results of the product inhibition studies and substrate inhibition by phosphate on Ade $\rightleftarrows$ MeSAdo are consistent with an ordered Bi Bi reaction with methylthioadenosine the first substrate to add to and adenine the last product to leave the enzyme. Michaelis constants determined for substrates of the enzyme were 8×10^{-6} M for methylthioadenosine and 13.5×10^{-3} M for inorganic orthophosphate.

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Molecular Toxicology

MECHANISMS OF TOXICITY, MUTAGENESIS AND CARCINOGENESIS BY METAL POLLUTANTS

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Certain metals are nutritionally essential for animals, including humans, yet are potentially toxic at high doses. Ever-increasing quantities and varieties of toxic metal compounds are now being released as pollutants, for example, from coal combustion, mining, asbestos production, etc. Although it is important to screen for potential toxicity of the many metal compounds thus released, it is even more essential that we understand the biochemical mechanisms leading to metal-induced toxicity in target tissues. We are attempting to

correlate our findings on toxicity with the physical and chemical properties of metals. We hope that such studies will be useful in setting acceptable limits for exposure to toxic metal pollutants.

The toxic effects of various metal ions are well known and a quantitative measure of their relative toxicity has been obtained through acute toxicity tests. One of our aims is to understand the basic mechanisms of metal toxicity using Drosophila and the mouse. The scope of this study may be seen from the five approaches that are being followed: (i) evaluation of Drosophila as a test organism for determining the acute and chronic effects of metal ions; (ii) determination of the effects of metal ions on proteins and nucleic acids in Drosophila; (iii) examination of the effect of metal ions on the structure of tRNA; (iv) measurement of mouse LD_{50} values for 19 metal ions under controlled, uniform conditions; and (v) development of a theoretical explanation for the differences in toxicity that exist for metal ions.

We have found that (i) among Drosophila strains the sensitivity to Cd^{2+} varies by more than a factor of 10. The lethal response of Drosophila to different metals generally parallels the response of mice. As Drosophila grow older they become more sensitive to the lethal effects of Cd^{2+}, with males more sensitive than females. (ii) The electrophoretic pattern of proteins changes progressively with age in adult flies, some proteins appearing and others disappearing. Sub-lethal concentrations of Cd^{2+} interfere with these changes where Zn^{2+} has no effect. (iii) The four tRNAs that contain queuine in the anticodon (Asn, Asp, His and Tyr) are markedly altered when the Drosophila are reared on media containing Cd^{2+}, Hg^{2+} or Sr^{2+}. (iv) Chromatography of Tyr-tRNA and Leu-tRNA on RPC-5 columns results in the resolution of 2 and 7 isoacceptors, respectively. The presence of Cd^{2+}, Zn^{2+} or Mg^{2+} during chromatography causes altered chromatographic behavior of each isoacceptor in a manner that is unique for each divalent cation. (v) Crystals of an adenine-cadmium compound were prepared and the structure was analyzed by X-ray diffraction. This is the first known crystal structure of a cadmium complex with a purine base. (vi) In a survey of the studies on the interactions of cadmium with proteins and nucleic acids (Jacobson and Turner, 1980), we argue that the theory of hard and soft acids and bases is especially useful in seeking an understanding of the interaction of metal ions with biological macromolecules.

Metals constitute an important class of mutagenic and carcinogenic pollutants. The mechanisms leading to malignancy or mutation are being studied by examining the effects of a number of metal compounds on DNA replication and transcription. Changes in the accuracy of replication and transcription in the presence of different metal ions are being investigated by measuring the degree of misincorporation of noncomplementary bases into DNA and RNA in both prokaryotic and eukaryotic systems. The rate of RNA chain initiation during transcription is being measured at various metal ion concentrations.

Twenty one metal salts (all as chlorides, except for sodium arsenite) were tested for their effect on rat liver DNA polymerase β in both inhibition of DNA synthesis and fidelity of replication using poly(dA-dT) as a template. The alkali metal ions were the least inhibitory, whereas Hg^{2+} was the most inhibitory. Surprisingly, Zn^{2+} and Cd^{2+} were comparable in their inhibitory capacity. Except for the alkali metals, all metal ions whether carcinogenic or not caused misincorporation (dCMP in place of dTMP), although at different concentrations depending on the metal. For example, both Zn^{2+} and Cd^{2+} increased misincorporation 10-fold at comparable concentrations (50-200 μM). These results are in sharp contrast to those of Sirover and Loeb who showed a positive correlation between metal-induced misincorporation into DNA and the carcinogenic potential of metal ions.

The fidelity of transcription has also been measured in the presence of certain metal ions, using poly(dA-dT) as a template. A greater than 3-fold increase in misincorporation is seen in the presence of 2 to 10 mM Mn^{2+} that is not reversed by higher concentrations of Mg^{2+}. Cd^{2+} even at very low concentrations (0.1 to 2 mM) strongly inhibits overall transcription and causes a concentration-dependent increase in misincorporation of CMP in place of UMP (10- to 30-fold at 1 mM Cd^{2+} concentration). This misincorporation can be partially overcome by higher concentrations of Mg^{2+}.

Studies of the effects of mutagenic and carcinogenic metal ions on RNA chain initiation have been performed at lower pH (7.2-7.4) and in the absence of mercaptoethanol such that the metal ion under investigation remained in solution. Under these conditions, overall transcription is inhibitied by much lower concentrations of the test metal ion. We are still able to obtain our earlier results showing that metal ions which are mutagenic or carcinogenic (Pb^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+} and Mn^{2+}) stimulate initiation of RNA synthesis at concentrations that inhibit overall RNA synthesis, whereas metal ions not in this category (Na^{+}, K^{+}, Li^{+}, Zn^{2+}, Mg^{2+} and Ca^{2+}) inhibit initiation at concentrations that inhibit overall synthesis. We are currently testing the idea that certain mutagenic and carcinogenic metal ions increase initiation sites on the DNA template. There appears to be a correlation between the biological effect of the metal ion and its "hard" or "soft" character.

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ENVIRONMENTAL INSULTS TO DNA

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The aim of our research is to reach a better understanding of how a variety of chemical-physical agents interact with DNA to produce toxic effects. From a basic knowledge of the chemistry of these interactions, it is possible to (i) develop better *in vitro* test systems for purposes of risk assessment, and (ii) arrive at a more scientific basis for toxicity assessment. The development of reliable *in vitro* test systems obviates the need for lengthy and time-consuming, whole animal studies. A wide variety of spectroscopic, chromatographic, and centrifugal techniques has been employed for purposes of quantitating the extent of damage to DNA following exposure to a particular agent. Of particular interest is the development of assays for quantitating damage at the one part per 10^5 level without resorting to radioactive labels. These conditions simulate those typically found under normal circumstances in nature. The following represents a cross section of current research being done on the analysis of DNA damage: (i) The covalent binding of the anti-tumor drug diammino *cis*-platinum(II) dichloride to DNA has been studied using gel permeation and ion exchange chromatography. Various chemical and enzymatic methods of hydrolysis have been employed to separate the platinum adducts from the nonreacted bases. The use of the radioactive isotope ^{195}Pt has facilitated these studies. (ii) The action spectrum for the photolysis of iodine from iododeoxyuridine containing DNA has been measured, and its analysis reveals energy transfer over approximately one base on either side of an IU residue. The frequency of chain breakage as measured in alkali is approximately 1 break per 6 iodines lost. (iii) The

fluorescence at 77°K has been used to monitor the binding of benzo[a]pyrene to DNA in mouse skin. The sensitivity of this assay is such that one lesion per 10^5 bases can be measured in 100 µg of DNA.

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IDENTIFICATION AND CHARACTERIZATION OF NEUROTOXINS FROM FOSSIL FUELS

Peter Pfuderer

We are attempting to characterize neurotoxins in fossil fuel fractions and effluents by (i) adrenergic and cholinergic inhibition tests, (ii) preparative gas chromatography to separate the toxins, and (iii) organism testing and biochemical testing to determine the mode of action. The screening encompasses all toxins which can be made water soluble, while the applications are intended to be applicable to man.

The cholinergic assay is an inhibition assay of cholinesterase. At present, we are using electric eel cholinesterase. The mammalian enzymes reportedly were isolated by use of the same affinity column as for the eel enzyme, implying that the active sites are very similar. The adrenergic assay is a test of the inhibition of transmission to a fish melanophore in isolated goldfish tails. Most phenols respond positively to the latter test, while some alkaloids are positive in the cholinergic assay. One potent toxin has been isolated from shale oil and characterized as to molecular weight and boiling point, but it has not yet been identified.

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Environmental-Cellular Interactions

CESSATION OF RESPIRATION IN ESCHERICHIA COLI AFTER FAR-UV IRRADIATION

P. A. Swenson

One consequence of far-ultraviolet (UV) (254 nm) radiation damage to the DNA of Escherichia coli is cessation of respiration. The shutoff, which occurs about 60 min after exposure to UV, is dependent upon protein synthesis and the recA$^+$ and lexA$^+$ gene products. Other so-called rec/lex responses caused by damaged DNA include error-prone repair of DNA (as indicated by an increase in mutagenesis) and induction of lambda prophage. Cessation of respiration is a unique rec/lex response in that it is associated with cell death rather than with cell survival and is regulated by cyclic AMP and its receptor protein. Our objective is to understand the genetic and biochemical bases of the shutoff process and its control. A biochemical change that accompanies respiration shutoff in irradiated cells is the complete loss of unaltered pyridine nucleotides to the suspending medium. The loss implies that induced changes have occurred in the surface membranes of the irradiated cells. Work is beginning on the electrophoretic analysis of inner and outer membrane proteins of irradiated and unirradiated cells. Recent studies show that genetic control of the respiration shutoff system is more complex than other rec/lex systems. A tif-1 (a recA$^+$ allele) strain that forms filaments at 42°C does not shut off after UV. A polA1 strain, deficient in DNA polymerase I, shuts off only at UV fluences giving very low survival. The ineffectiveness of post-UV treatments with cyclic AMP (which shuts off respiration more completely in wild-type cells) and with 5-fluorouracil (which maintains respiration in wild-type cells) shows that the shutoff mechanism in polA1 is different from that in the wild-type strain. All other rec/lex responses occur in these two mutant strains. These data provide further evidence that respiration shutoff after UV is not an SOS (emergency) response concerned with cell survival.

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REGULATION OF TRANSPORT SYSTEMS IN CULTURED MAMMALIAN CELLS

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Cells and whole organisms interact with their environment through their membranes, plasma membranes for cells and cellular epithelial membranes for the organism. As a protective measure, the lipid bilayer of the surface membrane serves as an effective barrier to most of

the polar compounds that may be dissolved in the aqueous environment in which cells live. As a corollary, the dissolved substrates required by cells or organisms either for maintenance or for growth are commonly taken up by specific transport systems. The latter, in turn, are well regulated in terms of numbers of transporters, the affinities of the transporters, or their degree of coupling to metabolic systems. Our group has been studying various aspects of transport regulation in mammalian cells in relation to the metabolic requirements of the cells or, in model epithelial systems, the requirements of the organism. Special emphasis is given to changes in these systems in response to growth stimuli, including the effects of tumor promoters. Our principal findings are:

Turnover of Na,K-ATPase in HeLa Cells. This is an essential enzyme whose activity is responsible for the maintenance of alkali cations in cells. Under conditions of stress (substrate deprivation by K^+ depletion or treatment of the cells with cardiotonic glycosides with digitalis-like action) the enzyme is induced. In the steady-state of growth, the enzyme is turned over three times per generation, i.e., for the growth of each new cell the cell surface complement of enzyme is synthesized four times and degraded three times. The quantity of enzyme per cell is regulated on the degradative side of turnover; when the enzyme is induced the rate of synthesis is not detectably altered, but the degradation rate constant is decreased.

5'-Nucleotidase in HeLa Cells. This surface enzyme, of uncertain function, is a marker for HeLa plasma membranes. To use it quantitatively, we have shown that its surface density is constant throughout the growth phases of the cells and that it is not affected by drugs or other stimuli that affect the specific transport systems. We have extensively characterized the enzyme's activity in HeLa cell membranes.

Endocytosis in HeLa Cells. This is a process whereby the cells take up fluid from the environment, together with whatever it contains. In HeLa cells, the volume of fluid taken up corresponds to about 3% of the cell volume per hour. The contents of the vesicles taking up this fluid are either regurgitated into the medium or delivered to lysosomes. In the lysosomes, the contents are either metabolized or expelled from the cells by exocytosis; the latter process has a half-time of about 70 h.

Na^+-dependent Amino Acid Transport in Mammalian Cells. Many amino acids can be concentrated in mammalian cells by coupling to Na^+ influx. The energy in the Na^+ electrochemical gradient derives from the activities of the Na,K-ATPase. We have shown that in quiescent human diploid fibroblasts, stimulated with excess serum back into the growth cycle, concentrative amino acid uptake is rapidly stimulated by an enhanced membrane potential creating an enhanced driving force on the Na^+. Similar responses are seen in mouse erythroleukemic cells in response to the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA).

Responses of Mouse 3T3 Cells to TPA. In this model system, the cells respond to TPA by a specific induction of the enzyme ornithine decarboxylase (ODC). We find this response to be Ca^{++} specific and are testing the working hypothesis that an early effect of TPA on sensitive cells is the release of intracellular sequestered Ca^{++}, raising the levels of free cytosolic (Ca^{++}); according to the hypothesis, high cytosolic Ca^{++} is a necessary, if not sufficient, precursor to the induction of TPA effects.

Hexose Transport in Mammalian Cells. This transporter is also inducible in cells deprived of the substrate glucose. Human diploid fibroblasts so induced also show a profound metabolic change in the reduction of activity of the glycolytic enzyme phosphofructokinase. As a consequence, much of the transported glucose is not extravagantly secreted as lactate but is routed through the hexose monophosphate shunt. The bioflavanoid quercetin has been

shown by others to inhibit "runaway" glycolysis in tumor cells by a mechanism postulated to be related to the activity of cellular ATPase. We find that quercetin can inhibit glycolysis simply by its profound inhibition of hexose uptake. In studies on hexose uptake in a hamster cell line, we have derived a number of genetic variants that are deficient in transport. In the most thoroughly studied of these, the deficiency is due to an altered affinity of the transporter for its substrate rather than a deficiency in the number of transporters. The deficiency is associated with an alteration in glycoprotein synthesis and appears to be related to a change in sialic acid incorporation into the cell surface proteins.

Na^+ Dependent Hexose Transport in Cultured Epithelial Cells. This concentrative system, again utilizing the energy stored in the Na^+ electrochemical gradient, serves the needs of the whole organism by taking up glucose from the intestinal lumen or recapturing it from the glomerular filtrate in the proximal tubule of the kidney. LLC-PK_1 cells, a cultured line from pig kidney, grow like other cultured cells without this transport system until they become confluent, when they then develop this differentiated function. We find that the development of this differentiated transport can be accelerated by cyclic AMP analogs and compounds like hexamethylene bisacetamide, an inducer of differentiation in Friend erythroleukemic cells. Such differentiation can be inhibited by the tumor promoter TPA. This system is under intensive study at present.

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THEORETICAL AND APPLIED CRYOBIOLOGY

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The research of the Theoretical and Applied Cryobiology Group is devoted to low temperature biology, to the permeability and osmotic responses of cells, and to the effects of temperature and the state of the aqueous environment on the cell surface. The successful freezing of complex and sensitive biological systems requires an understanding of the fundamentals of low temperature injury, but an attractive feature of cryobiology is that a given experiment often yields information of both basic interest and practical utility. With this in mind, we are pursuing fundamental cryobiological studies, using for the most part cells that are themselves of practical interest in biology and medicine, namely, mouse embryos, mammalian erythrocytes, human granulocytes, mouse thymuses, T cells, and tissue culture cells.

There are two major categories of freezing injury, and we are studying both. (i) Above a certain critical cooling rate, ice forms within cells and is generally lethal. (ii) Cooling at much lower rates can also be lethal, and here the injury is associated with physical-chemical changes in the aqueous liquid solution inside and outside the cells.

Intracellular Freezing. Numerical solutions to coupled physical-chemical differential equations allow one to predict the numerical value of the critical cooling rate. Three important parameters are the permeability of the cell to water, its temperature coefficient, and the temperature at which intracellular supercooled water can nucleate. The water permeability (L_p) of fertilized and unfertilized mouse ova has been found to be 0.43 μm/min atm at 20°C. The activation energies are 13 and 14.5 Kcal/mol, respectively. We have also obtained the surprising result that supercooled water in mouse ova in 2 M dimethyl sulfoxide remains unfrozen to about -40°C. Using these three values, the critical cooling rate predicted by the differential equations agrees well with that observed under a special low temperature optical microscope.

Solution Effect Injury. As aqueous solutions freeze, the electrolytes in the external solution progressively concentrate, and, if cooling is slow, the cells undergo progressive osmotic dehydration. It has been generally thought that slow freezing injury is the result of either excessive salt concentration or excessive cell shrinkage. It is also possible that cell survival is affected by the size of the unfrozen channels in the solution or, more precisely, by the fraction of solution that remains unfrozen at any temperature. The phase rule dictates that solution concentration and fraction unfrozen are reciprocally related and ordinarily inseparable. One can separate them, however, by suspending cells in ternary solutions of nonelectrolyte, electrolyte, and water which differ in total solute concentration but maintain a fixed ratio of nonelectrolyte to electrolyte. We have performed these experiments with human red cells in solutions of glycerol-NaCl-water. Samples were frozen to various subzero temperatures which were chosen to produce various molalities of NaCl (0.24 to 3.30) while holding the fraction unfrozen constant, or conversely to produce various fractions unfrozen (0.03 to 0.5) while holding the molality of salt constant. (Not all combinations of these values were possible.) The following general findings emerged. (i) Few cells survived the freezing of more than 90% of the extracellular water regardless of the salt concentration in the residual unfrozen 10%. (ii) When the fraction unfrozen exceeded 35%, the majority of the cells survived even when the salt concentration in the unfrozen portion exceeded 2 molal. (iii) Salt concentration exerted a major effect on survival only when the fraction unfrozen lay between 10 and 35%. To find a major effect on survival of the fraction of solution that remains unfrozen was unexpected. It may require major modifications in how cryobiologists view solution-effect injury and its prevention.

Hopefully, these fundamental studies will aid in the successful freezing of difficult biological materials. Several are under study. (i) A cryobiological analysis of human granulocytes has yielded a set of conditions that permit high survivals of cells at 0°C, but the cells are injured for they die after a short period of time at 37°C. (ii) We have conducted experiments on developing a method to assess the functional survival of frozenthawed fetal mouse thymuses. The assay involves transplantation of the organs into the athymic nude mouse, and on this basis survivals appear high. (iii) Cryobiological analyses of cloned cytotoxic T cells and their target cells and of precursor B cells have been conducted and have led to a set of procedures that yield high survivals after freezing and thawing.

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Protein Structure and Enzyme Mechanisms

DESIGN AND APPLICATION OF AFFINITY LABELS FOR THE CHARACTERIZATION OF ACTIVE SITES OF ENZYMES

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Active-site characterization is a prerequisite to acquiring a comprehensive understanding of enzymic mechanisms. The most versatile approach for the selective introduction of a chemical label into the active site, thereby providing structure/function correlations and identifications of active-site residues, is affinity labeling. In its traditional form, affinity labeling entails the use of reactive analogs of natural substrates to label substrate binding sites. Conceptually, it can be viewed as combining features of competitive inhibitors and general protein reagents into a single molecule. The substrate-like features of the reagent direct it to the active site in a fashion completely analogous to the binding of competitive inhibitors. This binding step results in a high localized concentration of reagent within the substrate binding site and thus increases the likelihood of modification of a residue within this site as compared with other positions of the protein molecule.

Much of our recent effort has been focused on ribulosebisphosphate carboxylase, the plant enzyme essential for the photosynthetic assimilation of CO_2. This enzyme also possesses inherent oxygenase activity which accounts for photorespiration, a nonessential, energy-wasteful process that reduces net CO_2 fixation. Thus, an understanding of the *in vivo* modulation of the carboxylase/oxygenase ratio and a determination of whether this ratio can be systematically manipulated by external means are of major agronomic significance.

Previously we were successful in designing two affinity labels for ribulosebisphosphate carboxylase/oxygenase. Although the reagents lacked absolute specificity, they permitted the identification of two cysteinyl and two lysyl residues in the active-site region. In an attempt to obtain reagents with improved specificity for the active site, we have prepared two reactive derivatives of the transition state analog 2-carboxyarabinitol bisphosphate, which binds noncovalently to the enzyme with a K_d of 10^{-11} M. The compounds prepared are the acid azide and the 3(4)-chloroacetyl derivative of carboxyarabinitol bisphosphate.

We are also determining the complete amino acid sequence of the carboxylase/oxygenase, which is essential to solving the 3D-structure by X-ray crystallography.

Another major effort involves the design of reactive analogs of α-ketoglutarate as potential active-site probes for the numerous enzymes that process this key metabolite. One such compound, (RS)-3-bromo-2-ketoglutarate, is a substrate, inactivator, and ligand for affinity chromatography of $NADP^+$-dependent isocitrate dehydrogenase.

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STRUCTURE AND FUNCTION OF THE AROM MULTIENZYME SYSTEM

Frank Gaertner K. H. Cole

Five of the enzymes in the central pathway leading to the biosynthesis of the aromatic amino acids in Neurospora crassa are known to be physically associated. Previously, it was thought that the enzymes of this system were joined by noncovalent forces in the form of a multienzyme complex. It was also thought that the five genes encoding the system in this eukaryote were uniquely organized in an operon-like unit that directed the synthesis of a polycistronic mRNA. In contrast to these notions, we have found that the five enzymes are components of a single polypeptide chain, and that the genetic system, rather than existing as an operon-like element, exists as a single gene encoding a single mRNA from which is translated a single 150,000 dalton polypeptide. The intact enzyme system functions as a 300,000 dalton homodimer. We have also found that this enzyme system, which we have termed a multienzyme conjugate, is highly sensitive to proteolysis by an assortment of proteases active in N. crassa. The function of these proteases and peptidases is not known, but we have been able to account for more than 25 distinct proteolytic activities, including acid, neutral and alkaline activities, serine proteases, trypsin-like and chymotrypsin-like activities, aminopeptidases, carboxypeptidases, iminopeptidases, and tryptophan and proline specific activities. Which of the many activities are involved in modifying the structure of the arom enzyme conjugate in vitro or in vivo is not yet known, but it appears that limited proteolysis may play a role in altering the function of the arom conjugate and may be involved in interrupting and diverting a metabolic channel.

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BIOORGANIC CHEMISTRY AND ENZYME CATALYSIS

D. G. Doherty

This program involves the application of synthetic organic chemistry to the solution of biological problems in the area of carcinogenesis and mutagenesis. In the carcinogenesis area, previous work in a human leukemic cell system by J. D. Regan established the importance of serine in metabolism and implicated the serine transhydroxymethylase system (STM) as the mediator. In a collaborative effort, we synthesized a number of compounds related to serine as possible enzyme inhibitors. The most effective one of these, 1-hydroxyaminocyclohexane carboxylic acid, has been prepared in quantity and submitted to the Drug Evaluation Branch of the National Cancer Institute for testing. They have recently reported it to be active against Leukemia P 388, and an additional quantity has been synthesized and supplied for further testing in other systems. Additional possible inhibitors of the STM system are in preparation. The identification of mutationally altered esteroproteases in mammalian tissues and electrophoretic gels by histochemical means depends upon the availability of suitable chromogenic substrates. Acyl naphthyl esters in the case of methionine, proline, and tryptophane have been shown to be useful because the napthol released can be coupled with a diazo compound to yield an intense color. However, none has been prepared from amino acids with functional side chains such as the basic and acidic amino acids and cystine. We have consequently prepared a series of new alpha and beta naphthyl esters from suitably blocked precursors for evaluation as indicators of variations in tissue esterases.

X-RAY DIFFRACTION

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The aim of our research is to determine by X-ray crystallography the three-dimensional structures of molecules of biological interest in order to lay the basis for understanding their functions on a molecular level. Our work with macromolecules has included, in almost every case, their isolation, purification, characterization, and crystallization prior to X-ray studies.

Proteins. During the past two years, protein work was concentrated on a soybean trypsin inhibitor, PI-I. X-ray data were collected from the native protein and a series of isomorphous heavy-atom derivatives. From the Fourier at 5.5-Å resolution, the molecular boundary was established, and the molecule appeared to have a multilooped structure, as expected from the presence of five intrachain disulfide bonds. A 3.2-Å Fourier has been calculated, but tracing of the peptide chain was hampered, apparently because of excessive radiation-damage effects. It appears necessary to re-collect the data with faster scans and less exposure per crystal.

Proteins newly crystallized in usable size included the Bowman-Birk inhibitor from soybean seeds and cardiotoxin III, one of four cardiotoxins separated and purified from venom of the Taiwan cobra. However, cardiotoxin III diffracts poorly.

A new phasing formula (described previously) for the multiple isomorphous replacement method was shown to give better results than the usual Blow-Crick formula when applied to test data containing different sets of random errors.

Small-molecule Structure Determinations. The first known X-ray structure determination of a complex of cadmium (a carcinogen) with a purine base is that of cadmium adeninium nitrate monohydrate. The structure of bisadeninium dinitrate monohydrate exhibits adenine-adenine pairing like that of poly(rA). We have also determined the structure of bulbocapnine, an alkaloid, and of a crystal of unknown composition, shown to be sodium methylsulfonate.

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PROTEIN LUMINESCENCE

J. W. Longworth

Proteins luminesce from their constituent tyrosyl and tryptophyl residues when excited with short wavelength ultraviolet radiation. These aromatic moieties both absorb and emit radiation through spectrally distinguishable bands. Protein emission is dominated by tryptophyl contributions, and this is very diverse in spectral location, lifetime, and yield. Excited tryptophyl interacts strongly with its environment and in a protein each residue is surrounded by distinct groupings of amino acids which create a diversity of environment. My interest in luminescence spectroscopy of proteins is the detection of this inherent heterogeneity through spectroscopy, the assignment of spectral components to specific residues, and the explanation of the observed spectral behavior through the chemical nature of the environment. Two aids in this endeavor are the fluorescence lifetimes, which can be crucial in decomposing spectral heterogeneity, and a comparison of divergently evolved proteins that have different amino acid sequences yet still maintain homologous patterns of folding of the amino acid chain, thus modifying in a known way the environment of luminophoric residues.

The diverse chemical environment of tryptophyl is not the entire basis for interpreting protein fluorescence. Excited singlet state of tyrosine is a much stronger acid than its ground state and it emits in the same spectral region as tryptophyl residues. The excitation spectrum of this photoionized emission is that of tyrosine, and this can be used to disclose the presence of appreciable tyrosinate fluorescence in human and bovine serum albumin. The formation of ionized tyrosine fluorescence is largely absent in denatured albumin, suggesting that interior tyrosines are the source of this radiation. A possible H-bonded partner is the side chain ε-amino group of lysine. An internal H-bond between tyrosine phenoxyl and lysine ε-amino groups is found in lobster glyceraldehyde-3-phosphate dehydrogenase. This tyrosine is absent in the enzyme of Bacillus stearothermophilus. Published fluorescence spectra show the presence of an additional long wavelength component in rabbit enzyme which probably has this H-bond complex. The complication is the presence of an additional tryptophyl in eukaryote enzymes though this residue is involved in the subunit contacts and may fluoresce at short wavelengths.

Earlier studies of the fluorescence of kappa light chains of immunoglobulins, which are fluorescent from a single tryptophyl residue located in the interior of the variable domain, showed the great diversity of spectroscopic behavior. In addition now, some caution needs to be taken in assuming that there is no contribution from photoionization of tyrosyl residues, and that the fluorescence is attributable to tryptophan. There are sound, but circumstan-

tial, arguments to suggest that the source of fluorescence of light chains is from a single tryptophyl residue. This is buried in the interior core of the variable domain, complexed with a water molecule, and surrounded by hydrophobic side chains. These side chains are highly conserved between different sequences, and any variation in the chemical surroundings must be small and quite subtle. Only small variations in the interactions with a disulphide linkage and an exciplex with water appear permissible, but this is sufficient to create extreme spectral variability. Thus I have shown that the relationship between protein structure of tryptophyl fluorescence properties is surprisingly subtle, and it is only rarely that specific complexes like the tyrosyl-lysyl H-bond complex can be specified.

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Cellular and Comparative Mutagenesis Section

Section Overview

The Cellular and Comparative Mutagenesis Section contains a great diversity of research, but has a common goal of providing information relevant to the estimation of the genetic hazards of radiation and chemicals to man, especially as these biohazards are encountered in energy-related processes. Our strategy is to expand our understanding of the mechanisms of mutagenesis and to develop and validate a variety of test systems for assay of potentially hazardous agents.

The Section is divisible into four research units, which, of course, have considerable overlap as well as interactions with other groups within the Biology Division and other divisions of the Oak Ridge National Laboratory. These research units are Comparative Mutagenesis, Mammalian Cellular and Molecular Studies, Genetics of Microorganisms and Drosophila, and Development and Reproductive Physiology. In each of these units there are research projects designed to provide a better understanding of the mechanisms of mutagenesis and also the utility of assays developed here or elsewhere. If these studies are to provide information to aid in the protection of the occupationally and generally exposed public from genetic hazards and to make possible the prediction or even reversal of such damage, more must be learned about the way in which genes act and are expressed. Thus, continuing and parallel research yielding knowledge of genetics and reproduction in a variety of organisms, including man, must accompany and/or underlie the development of test systems.

There are also inter- and intradivisional programs and interactions that involve many facets of the Cellular and Comparative Mutagenesis Section, for example, the recently instigated Biotechnology Program and the health effects research project within the Life Sciences Program in synthetic fuels. Furthermore, the Section contributes to graduate training in genetics within the University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences.

The above represents a summary of the organization of the Section. However, these divisions are very flexible when the research interests are considered. In the area of genetic toxicology the main effort is the development and validation of assays for mutagenicity and teratogenicity. When coupled to the efforts in the other Sections, the array of bioassays represents a rather complete coverage of testing procedures in genetic toxicology.

The assays encompass bacteria (DNA repair and mutagenesis in Salmonella, E. coli, and Haemophilus), fungal systems (mutagenesis in yeast and Neurospora), Drosophila (sex-linked recessive lethal tests, non-disjunction, chromosome damage, and sister chromatid exchanges), amphibia (teratogenesis and embryo toxicity), mammalian cells *in vitro* (mutagenesis, chromosome aberrations, DNA repair, and sister chromatid exchanges), and mammalian cells *in vivo* (chromosome aberrations, mutagenesis, and sister chromatid exchanges). There is also an interaction with the Comparative Animal Research Laboratory where mutagenicity assays involving several plant systems are being developed. The individual research summaries provide details of these various research programs. The point to be emphasized here is that information from short-term and longer-term assays using a wide variety of different systems will provide a basis for extrapolation to potential effects in man.

Several aspects of the program are involved rather directly with extrapolations to man. The most direct approaches are naturally those involving studies on mammalian systems, for example, in vivo/in vitro cytogenetics and mutations assayed in mammalian hemoglobins. Other less direct approaches involve comparisons between Drosophila and mouse including those obtained from inhalation studies and from the mutagenic effects of diesel fuels. Since it is possible that mutagenicity is predictive of carcinogenicity (a correlation being studied by a number of investigators within the Section), it is hoped that mutagenicity assays can be used to predict the potential carcinogenicity of a compound to man. Finally, the Development and Reproductive Physiology Unit is involved in a variety of studies concerned with the teratogenicity and embryo toxicity of pollutants in amphibian systems. Validation of these end points as compared to mammalian systems could lead to the prediction of similar effects in man.

In order to better interpret the data from these various assays and to extrapolate to effects in man, it is essential that the underlying mechanisms of mutagenesis, carcinogenesis, teratogenesis, and clastogenesis be determined. It is clear that DNA repair is involved in the process of mutation induction, and a comprehensive study of the enzymology of repair is being carried out. Under this category is included the collaborative intersectional efforts in the area of photobiology. Detailed studies of recombination and gene expression in Drosophila will provide basic information towards understanding mutation induction.

Other mechanistic approaches involve the study of metabolic activation, whereby nonmutagenic compounds are metabolized into mutagenic forms, and the study of the relationship between the structure and mutagenic potential of related compounds, e.g., nitrosamines, polycyclic aromatic hydrocarbons, and aromatic amines.

An end-product of mutagenesis research is information from which regulatory guidelines can be established. Several projects directly impinge upon regulatory guidelines, including an interdivisional project concerning the applicability of short-term tests and chemistry to the Resource Conservation and Recovery Act. In addition to contributing to the data base useful to the Environmental Protection Agency under the Toxic Substances Control Act, the Section has coordinated the "GENE-TOX" activity for the Office of Toxic Substances. The Section has also cooperated with EPA in research on potential genetic effects of diesel fuel emissions. Much of the developmental work on the use of short-term assays in the evaluation of health effects of coal conversion and shale oil technologies has been carried out within the Division under Interagency Agreements between the EPA and DOE. These contributions stand not only as examples of how our long experience in fundamental research can be applied to the problems of modern technology but also as examples of cooperation between teams of investigators in various divisions of ORNL.

The following summaries will indicate how individual research projects fit into the above statement of purpose and how the information obtained might be used to estimate genetic effects on man from environmental agents.

Comparative Mutagenesis

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The Use of Short-term Tests in the Isolation and Identification of Chemical Mutagens in Complex Mixtures. The feasibility of using short-term mutagenicity assays to isolate and identify the potential biohazard(s) of complex materials is being examined by use of various coupled chemical and biological approaches. Such research has usually involved a preliminary chemical characterization and preparation for bioassay, followed by testing for bioactivity (generally the mutagenicity test for Salmonella histidine reversion described by Ames). Subsequent fractionation procedures to further characterize the mutagens present are carried out, with the bioassay being used as a tool to follow the activity and guide the separations. The mutagenicity tests are intended to function as (i) predictors of profound long-range health effects such as mutagenesis and/or carcinogenesis; (ii) a mechanism to rapidly isolate and identify a hazardous biological agent in a complex mixture; and (iii) a measure of biological activity, correlating baseline data with changes in experimental (or environmental) conditions and, in the case of actual industrial effluents or streams, with changes in process conditions. With this combined chemical fractionation and short-term assay approach, information is being accumulated on the actual compounds responsible for the biological effect. Thus, the mutagenicity tests will also function as (iv) an aid in identifying the specific hazardous compounds involved and in establishing priorities for more definitive chemical analysis and monitoring along with further validative testing in comparative systems, including whole-animal testing, for mutagenesis and carcinogenesis.

Although our work has emphasized evaluation of test materials from the developing synthetic fuel technologies, the procedures are generally applicable to a wide variety of industrial and natural products, environmental effluents, and body fluids.

Short-term tests with bacterial and fungal mutagenicity assays appear to detect effectively the mutagenic potential of complex environmental or industrial effluents; however, chemical fractionation is necessary to reduce toxicity and concentrate hazardous materials. Extension of the results to higher organisms, i.e., mammalian cells, Drosophila, and the mouse, appears to be valid but needs more testing.

Use of Bacteria in Mutagenicity Testing. We are developing short-term bacterial assays for mutagenicity testing and also applying the assays developed by B. N. Ames (Salmonella typhimurium) and G. R. Mohn (E coli K12, 343/113) to chemicals of interest.

In order to determine the empirical relationship between mutagenesis and carcinogenesis, we have assayed a large number of nitrosamines that have been characterized in whole animal carcinogenesis studies by W. Lijinsky, Frederick Cancer Research Center. In general, carcinogenic cyclic nitrosamines and their derivatives were mutagenic while noncarcinogenic derivatives were not mutagenic in the Salmonella histidine reversion assay and E. coli arginine reversion assay. The aliphatic nitrosamines failed to show any such relationship. In all of these assays metabolic activation with liver preparations from phenobarbital-induced Sprague-Dawley male rats was incorporated. Use of the E. coli assay complemented the

Salmonella assay, and in a few cases false negatives in the Salmonella assay were picked up as mutagens with E. coli (e.g., nitrosomethylethylamine). The study with nitrosamines was also useful in determining chemical structure-biological activity relationships. Substitution in the alpha position next to the N-nitroso group of cyclic nitrosamines has eliminated both mutagenic and carcinogenic activities, suggesting involvement of that position in metabolic conversion of cyclic nitrosamines. Several derivatives of metaphenylenediamine that are of importance to the hair-dye industry gave similar results. A substitution in the α-position to the amino group abolished the mutagenic activity.

Similar chemical structure - mutagenic activity relationships were determined using a large number of aromatic hydrocarbons. An enhancement in mutagenic activity was observed with an increase in complexity of aromatic ring structure. Heterocyclic compounds were more mutagenic than the parent hydrocarbons. Substitution with amino or nitro groups of the aromatic hydrocarbons greatly enhanced mutagenic activity.

The bacterial mutagenicity assays were also utilized to examine the effects of known co-carcinogens and tumor promoters on mutagenesis. Several co-carcinogens were found to enhance mutation induction (co-mutagens) in Salmonella (e.g., benzo[e]pyrene, pyrene, anthracene, chrysene, naphthalene, etc.). However, promoters such as phorbel esters (12-O-tetradecanoylphorbol-13-acetate) were ineffective as co-mutagens. Saccharin, which many recent studies have implicated as a promoter, was not co-mutagenic in the Salmonella assay.

Certain polycyclic aromatic hydrocarbons (anthracene, pyrene, etc.) were not mutagenic in the Salmonella assay. When anthracene or pyrene administration (50 μg/plate) was followed by exposure to near UV, a dose-dependent cytotoxicity was noted with no apparent induction of histidine reversion. The strains with UV excision repair exhibited greater resistance to cytotoxicity suggesting damage to DNA as a cause for the cytotoxicity. These results are of clinical importance for the use of polycyclic aromatic hydrocarbons such as anthracene or coal-tar constituents in the treatment of psoriasis.

Chemical Mutagenesis in Yeast. We have been using yeast (Saccharomyces cerevisiae) as a model eucaryotic system to study the general nature of genetic change and to examine the mutagenic effects of chemicals as a source of induced genetic variability. Our basic programs have emphasized two approaches to chemical mutagenesis: (i) development of yeast strains and assay methodology suitable for chemical mutagenesis research, and (ii) definition of structure-activity relationships within families of chemical homologs.

Test development work has focused on validation of the can^r - HIS^+ forward and reverse mutation assay scheme, using an homologous series of amino and nitro aromatic compounds of known activity. Recently we have begun to extend this work into a series of N-heterocyclic compounds as well. In the interest of improving the resolving power of the assay for frameshifting mutagens, new markers for a frameshift reversion assay are being characterized. This work is being done in collaboration with J. F. Lemontt. A new line of investigation has also been opened in characterizing the endogenous mutagen metabolizing capacity of yeast. Activation of halo, amino, and nitro aromatics has been accomplished using log-phase cultures. A detailed examination of yeast-mediated activation of nitrosamines is being pursued.

Structure-activity research with yeast has emphasized several homologous series of cyclic nitrosamines differing in ring size, hetero atom composition, and position and type of substituent groups. Mutagenesis studies with nitrosated piperidines, piperazines, and pyrrolidines have emphasized the unusual reactivity of the α carbon atoms. Halogenation also

appears to contribute to electronic induction effects determining mutagenic potential. A novel activation mechanism involving a nitrosamine utilized as an illicit substrate of a normal yeast enzyme is under investigation. Further structure-activity studies with dialkyl nitrosamines are being initiated.

Chemical Mutagenesis in Drosophila melanogaster. The advantages of using Drosophila in the detection of mutagenic activity of various chemical and physical agents are well established. Our laboratory has taken a multifaceted approach in the use of Drosophila in the comparative mutagenesis program. (i) Using model pure compounds and selected fractions of complex mixtures, we have been able to correlate mutagenic activity in Drosophila with that obtained in other mutagenicity assays. In addition we have investigated the relationship between chemical structure and mutagenicity in a series of nitrosamines. (ii) The availability of mutants deficient in various steps of DNA repair has allowed us to begin characterization of these mutants with respect to the effect of selected classes of chemical compounds on mutagenicity. Efforts to isolate and characterize the microsomal system of Drosophila were initiated and in collaboration with L. Waters, we are investigating the metabolism of promutagens in Drosophila. This will allow us to compare such metabolism in insects and mammals as well as to detect differences in metabolic activity between various mutants of Drosophila. (iii) C. McKinley and Ti Ho have developed an *in vivo* assay in Drosophila for sister chromatid exchange (SCE). This offers an excellent opportunity to examine the relationship of SCE to other genetic end points. These efforts will lead to a better understanding of the mutation process in Drosophila as well as increase the utility of the Drosophila mutagenicity assays in testing programs.

a. Specific compounds assayed in a comparative approach. Because of the impact of numerous chemical insults on the health of man and his environment, many biological assay systems have been developed in order to evaluate potential health effects. In our laboratory we have demonstrated the utility of our existing Drosophila assay, SLRL, in the evaluation of the mutagenic potential of numerous pure chemicals and complex mixtures.

The nitrosamines were selected as model compounds for the comparative studies because of extensive data on their carcinogenicity. Mutagenicity testing of the cyclic nitrosamines showed that structural changes that modified the carcinogenic effect also modified the mutagenic response. Substitution on the carbon atom alpha to the nitroso group decreased or eliminated the mutagenicity; this is also the case with carcinogenicity.

In addition to the cyclic nitrosamines, a series of aliphatic nitrosamines that had been previously tested in the Salmonella/mammalianmicrosome assay were examined for mutagenicity. A total of 15 compounds were tested, 12 of which were listed as weak to fairly strong carcinogens and 3 as noncarcinogens. Of the 12 carcinogens, 9 that were moderate or strong were all mutagenic. Only 1 of the weak carcinogens and 1 of the noncarcinogens were mutagenic. Many of the compounds that were carcinogenic, but nonmutagenic in the Salmonella assay, were detected as mutagens in Drosophila. The three weak carcinogens were re-examined in Drosophila using an excision-repair-deficient strain, and all three were found to be mutagenic.

b. Development of bioassays. In addition to our interests in demonstrating the utility of the sex-linked recessive lethal (SLRL) assay of Drosophila in a comparative mutagenesis program, we have initiated experiments designed to increase the sensitivity of the assay, and we have begun to focus our attention on the characterization of the Drosophila microsomal

system. These efforts will allow us to better utilize Drosophila in mutagenicity assays and gain further insight into mechanisms of mutagenesis in Drosophila.

Increasing the sensitivity of the SLRL assay: That the SLRL assay of Drosophila is a sensitive indicator of genetic damage by chemical and physical agents has been repeatedly demonstrated although there are certain classes of compounds, such as the aromatic amines and PAAs, that are ineffective. We are attempting to characterize the response of repair-deficient mutants to a variety of chemical mutagens. Mutants isolated in other laboratories as mutagen-sensitive strains have been shown to be defective in DNA repair by Boyd and his co-workers. Since the sensitivity of the Salmonella test is increased by the incorporation of repair-defective mutants, we reasoned that it might also be the case with Drosophila. Initial experiments have indicated that the sensitivity in Drosophila can be increased by utilizing repair mutants but that the response is mutagen as well as gene specific. For these reasons we feel that many more pure chemical compounds, as well as alleles, must be tested before the repair-defective strains can be incorporated into a routine testing system.

Drosophila microsomal system: Recent discoveries that indirect mutagens and carcinogens require activation by the microsomal enzyme systems add even another dimension to the utility of Drosophila as a mutagenic test organism. In recent years considerable attention has been focused on the metabolism of certain drugs and pesticides by insects. It has been shown that the crucial step in such metabolism is oxidation by MFO which can be isolated as a microsomal fraction.

Our laboratory has developed procedures for the isolation of Drosophila microsomes in an attempt to characterize the Drosophila activation system. Microsomes are assayed for activity using the Salmonella test system. We have tested 2-acetylaminofluorene (2-AAF), 2-aminofluorene (2-AF), 2,7-diaminofluorene (2,7-diAF), 2-aminoanthracene (2-AA), 1-aminoanthracene (1-AA), 1-aminopyrene (1-AP), pyrene, benzo[a]pyrene (BP), 1,2,3,4-dibenz[a]anthracene (1,2,3,4-diBA) and aflatoxin B1. All gave a positive response except 1-AA, pyrene, BP, and 1,2,3,4-DiBA. Other pure compounds, in particular the nitrosamines, are currently being investigated.

In addition to pure compounds, we have tested the ability of isolated Drosophila microsomes to activate selected test fractions from Synfuel B. Three of the basic fractions were tested and all three were activated by Drosophila microsomes. The highly active (Salmonella) basic fraction was more mutagenic with Drosophila microsomes than with rat-liver microsomes.

c. In vivo induction of sister chromatid exchange (SCE) in Drosophila. The ability of the SCE assay to detect low levels of mutagens and carcinogens is well established. In order to correlate SCE with other widely used measures of mutagenesis, such as SLRL, dominant lethals, chromosome aberrations, and micronuclei formation, we have applied this technique to Drosophila. A battery of excision and postreplication repair-deficient mutants will be included in these studies to examine the relationship between DNA repair and SCE formation.

Although it has been shown both genetically and cytologically that spontaneous SCE is rare in Drosophila, induction of exchanges by substances mutagenic in the SLRL test has not been investigated. To date we have tested SCE induction in Drosophila with EMS, MMS, cyclophosphamide, DMN, nitrogen mustard and mitomycin-C. Good differential staining of chromatids was obtained and exchanges are easily scored in larval ganglia cells. Preliminary results indicate frequencies of induced SCE 4-10 times higher than spontaneous with low concentrations of DMN, MMS, and mitomycin-C. With the mutagens examined, concentrations which induce significant levels of SLRL also induce measurable levels of SCE in Drosophila.

Experiments are under way to examine correspondence among dose, SLRL frequency, and SCE frequency.

Cytogenetic Studies of Potential Mutagens in Human Leukocytes. A goal of this laboratory is to provide a realistic risk-to-human assessment of the chemicals present, or potentially present, in the environment. As part of our continuing effort to evaluate and upgrade these test systems, we have investigated the cytogenetic effect of various potential mutagens on human leukocytes. These results were then compared with the biological effects measured in other test systems.

One part of this study was to measure the effects of the industrial compounds, methylene dianiline (MDA), meta-phenylene diamine (MPDA), 4-4'-methylene-bis-2-chloroaniline (MOCA), and 4,4'-methylene-bis-2,6-diisopropylaniline (MDiPA). In Salmonella histidine reversion, all the compounds except MDiPA are mutagenic and require microsomal activation. In yeast mutation tests, all the above compounds are mutagenic; microsomal activation does not influence the chemical's effect. In Drosophila, none of the chemicals produce SLRL; Drosophila microsomes induced chemical activation in the Salmonella test system. Cytogenetic tests were performed with and without metabolic activation on human leukocytes in vitro. Chemical effects were determined by detecting chromatid aberrations and SCE. Positive controls were carried out with cyclophosphamide, requiring activation, and triethylene melamine, direct acting. Results show that none of the potentially mutagenic industrial compounds produce significant cytogenetic effects. Also the response was not enhanced by pretreatment with enzymatic activation. Thus, we concluded that the tested agents have shown mutagenic effects in lower systems but do not necessarily affect higher organisms.

In another study, a number of cyclic nitroso compounds selected on the basis of their mutagenic response and requirement for activation in submammalian assays were screened for the induction of SCE in human leukocytes. Two groups are included: (i) N-nitrosopiperidines (NPD), and various substituted nitrosopiperidines, and (ii) N-nitrosopiperazine (NPZ) and several nitroso-derivatives. Metabolic activation with rat-liver microsomal preparations was considered. The parental NPD and the chlorated and methylated derivatives of both NPD and NPZ gave a positive response. The oxygenated and carboxylated derivatives caused only a slight increase in the number of SCE, results of parallel investigations with bacteria, yeast, and Drosophila showed a good correlation between SCE, mutagenesis, and carcinogenesis. However, exceptions exist from system to system and the study demonstrates the efficacy of a battery of assays when considering the biohazard(s) of any group of compounds.

Metabolic Activation of Mutagens: Comparative Studies. Most chemical mutagens must be metabolized to some extent to be mutagenic. Identification of the active metabolites and the pathways by which they are generated will be useful in evaluating mutagen test systems and in making valid risk-to-human assessments. Our approach is to study the metabolism of selected mutagens in organisms that are both sensitive and resistant to their mutagenic effect. From this comparative approach we expect to learn more about how chemicals are activated to mutagens specifically and more about the entire process of mutagenesis generally.

A battery of tests is available in the Comparative Mutagenesis Section to measure the mutagenicity of suspect chemicals. These include the Ames Salmonella assay that can be coupled to microsomal-activation systems derived from a variety of sources, a yeast system, the Drosophila SLRL test system, and a variety of systems for measuring DNA damage as indicated by sister chromatid exchanges. Previous studies have indicated that these test systems can differ greatly in the efficiency with which they detect a mutagen. Possible reasons for these differences include: (i) different rates or extents of metabolic

activation, (ii) efficiency with which the active metabolites interact with sensitive sites in the DNA, and (iii) the rate at which the test organisms can repair the damaged DNA.

In only a few cases is the active metabolite of a mutagen known, or even inferred. Often this information was obtained by studies using only one — or a few closely related — species and whether extrapolation to other organisms, particularly human, is justified is uncertain. We believe that a comparative approach to the study of metabolic activation, utilizing a variety of organisms, will be useful. In addition to providing valuable basic information regarding the molecular biology of mutagenesis, these studies will surely indicate whether mutagen activation occurs via universal mechanisms or not - an important practical consideration in making human risk assessments.

Nitrosamines were chosen for these studies for reasons that include the following: (i) We have an ongoing collaboration with W. Lijinsky and co-workers who synthesize and test the carcinogenicity of the compounds. (ii) Nitrosamines are potent mutagens in Drosophila, a system in which activation and mutation frequency can be easily studied in the same organism and in which the activating system should be amenable to genetic manipulation. (iii) Nix and Rao have noted striking differences in the relative mutagenicity of certain nitrosamines in Drosophila versus the rat-liver microsome-activated Salmonella assay. Preliminary results of studies with DMN can be summarized as follows: (i) In the Salmonella assay Drosophila microsomes are more effective than rat-liver microsomes in activating DMN to a mutagen, and (ii) DMN-demethylase activity (catalyzing the presumptive first step in DMN activation) is easily measured in rat-liver microsomes but is as yet undetectable in those from Drosophila. Whether this is indicative of a fundamental difference in activation pathways or is a reflection of technical difficulties remains to be determined. We are now prepared to correlate enzyme activities, cytochrome P450 contents, and mutagenicity in the Ames assay. With these capabilities, we expect to determine the molecular basis for the differences in mutagenicity of various chemicals in the various test systems. Results of these studies should: (i) add to our basic knowledge of mutagenesis, (ii) indicate the molecular basis for the strengths and weaknesses of the various short-term mutagenesis assays, and (iii) perhaps yield information that is useful in making risk assessments in humans.

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Mammalian Cellular and Molecular Studies

MAMMALIAN CELL GENETIC TOXICOLOGY

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Our goal is to employ mammalian cell culture systems to study the potentially mutagenic and cytotoxic effects of energy-related agents, leading to an eventual estimation of their risks to humans and the environment. We have employed the Chinese hamster ovary cell/hypoxanthine-guanine phosphoribosyl transferase (CHO/HGPRT) system to quantify the cytotoxicity and mutagenicity of various classes of promutagens and direct-acting mutagens. These include alkanesulfonates, alkylsulfonates, nitrosoguanidines, nitrosoureas, heterocyclic mustards, platinum(II)chloroammines, quinolines, nitrosamines, haloethanes, polyaromatic hydrocarbons, and subfractions of coal-liquified crude oil. Such studies also further validate the relationship between mammalian cell mutagenicity and animal carcinogenicity tests. In addition, the quantitative nature of this system provides a basis

for studying the interrelationship of mutagen-induced DNA lesions and gene mutation by nitrosoureas and platinum(II)chloramines, and the role of DNA replication and protein turnover for the expression of 6-thioguanine resistance phenotype after mutagenesis with ethyl methanesulfonate. As our development of a Multiplex CHO Genetic Toxicology System progresses, we have extended our studies to include determinations of chromosome aberration and sister chromatid exchange. Similar studies will be conducted as we develop a CHO/human cell hybrid system for determination of chromosomal deletion and loss. Our studies using the CHO/HGPRT assay, CHO Multiplex System, and the CHO/human cell hybrid permit determination of multiple, distinct biological effects leading to reliable identification of environmental agents associated with both cancer and genetic diseases.

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MAMMALIAN CYTOGENETICS

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The overall objective of the research in the Mammalian Cytogenetics Group is to provide estimates of the genetic hazard of radiation and chemicals to man, using cytogenetic end points to extrapolate from data obtained in laboratory animals to man. At the same time it is our intention to develop new or better assays for predicting the mutagenicity of chemicals and to determine the mechanisms of induction of chromosome aberrations and sister chromatid exchanges (SCE) by radiation and chemicals. The systems currently in use are human lymphocyte cultures, mouse somatic and germ cells (treated *in vivo*), male marmoset germ cells, mouse early embryo stages, and Chinese hamster tissue culture cells.

An analysis of reciprocal translocations, induced in mouse spermatogonial stem cells following X-ray exposures at low dose rates, showed that the frequency of translocations was reduced as the dose rate was decreased. The yield at 0.001 R/min was essentially the same

as at 0.003 R/min indicating that there was no increase at very low dose rates. The yield/cell/R at the lowest dose rate (0.001 R/min) was about one-sixteenth of the yield at 100 R/min. A similar experiment on a smaller scale is currently in progress with male marmosets.

A study is also under way to determine whether DNA damage induced by X rays in post-spermatogonial cells that is converted into chromosome aberrations is repaired before or after fertilization. The approach is to use fractionated exposures, with different time intervals between the doses, and to analyze cells at the first cleavage division. If the yields from the split dose are the same as for the same dose given as a single exposure, it can be concluded that repair has not taken place, whereas a decreased yield following the split dose would indicate repair. To date it appears that repair does not take place until after fertilization.

The mechanism of induction of chromosome aberrations by X rays and chemicals is also being studied. Use is made of a novel technique developed here in which cytosine arabinoside is used to inhibit DNA repair and allows strand breaks from repairing regions to be accumulated. On reversing the inhibition with deoxycytidine, the strand breaks can be converted into aberrations, giving greatly increased aberration frequencies. The main conclusions at this point are: (i) X-ray-induced aberrations result from the misrepair of base damage and not from directly induced strand breaks. (ii) The increased sensitivity of Down lymphocytes to X-ray-induced chromosome aberrations is due to a more rapid repair of the DNA damage leading to aberrations in Down cells than normal. (iii) Chemical clastogens can induce chromosome aberrations in all stages of the cell cycle and cannot be thought of as S-phase dependent. (iv) The DNA damage induced by chemicals (e.g., 4NQO) can interact with DNA damage caused by X rays to give yields higher than additivity. All these conclusions are important when considering the potential hazard of chemicals and radiation to man.

We are also initiating SCE assays, with particular emphasis on <u>in vivo</u> exposures. At this point we are developing a comparative study of chemically induced SCE in bone marrow, spermatogonia, and early cleavage embryos. The purpose is to have available a very sensitive assay to compliment the chromosome aberration assays already in use. At the same time we are studying the mechanism of induction of SCE as this is important in aiding the interpretation of results on induced SCE. It appears from preliminary experiments that SCE are the result of replication errors and do not result from postreplication repair of DNA damage.

This is, in very general terms, the scope of the Mammalian Cytogenetics Group. We continue to try to develop and utilize chromosomal assays and at the same time attempt to understand the mechanisms of induction of both aberrations and SCE. This approach should enable us to make reasonable estimates of the potential genetic hazards of both radiation and chemicals.

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DNA REPAIR IN HUMAN CELLS

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Our primary objective is to elucidate the molecular events in human cells when cellular macromolecules such as DNA are damaged by radiation or chemical agents. We study and characterize (i) the sequence of DNA repair events, (ii) the various modalities of repair, (iii) the genetic inhibition of repair due to mutation, (iv) the physiological inhibition of repair due to mutation, (iv) the physiological inhibition of repair due to biochemical inhibitors, and (v) the genetic basis of repair. Our ultimate goals are to (i) isolate and analyze the repair component of the mutagenic and/or carcinogenic event in human cells, and (ii) elucidate the magnitude and significance of this repair component as it impinges on the practical problems of human irradiation or exposure to actual or potential chemical mutagens and carcinogens.

The significance of these studies lies in (i) the ubiquitousness of repair (most organisms, including man, have several complex repair systems), (ii) the belief that mutagenic and carcinogenic events may arise only from residual (nonrepaired) lesions or that error-prone repair systems may be the major induction mechanisms of the mutagenic or carcinogenic event, and (iii) the clear association of repair defects and highly carcinogenic disease states in man [xeroderma pigmentosum (XP)].

Normal human cells are able to repair ultraviolet (UV) light-induced pyrimidine dimers in their DNA while cells derived from patients with the genetic disease XP show defective or reduced repair. The number and kinds of pyrimidine dimers formed in DNA by UV light are related to the base ratio, the wavelength, and the total exposure. Normal human cells can excise $\sim 10^6$ dimers within a 24-h period after 254 nm of simulated sunlight irradiation. At low doses of UV light, nearly all dimers are excised. At higher doses, dimer excision is inhibited. Arabinofuranosyl cytosine (ara-C) at 20 μm concentration in the presence of 2 mM hydroxyurea inhibits steps in DNA excision repair following UV irradiation and chemical insult. It causes gaps to accumulate in incised regions which can be observed on alkaline sucrose gradients. Whereas cells from XP patients show a decrease in strand interruptions following UV irradiation in the presence of ara-C (reflecting their lower excision repair

capacity), cells from variant lines of XP show an increase in strand interruptions relative to normal human cells. Studies carried out in our laboratory have been aimed at elucidation of the mechanism by which ara-C inhibition acts. We are currently evaluating the possibility that the dose-dependent nature of ara-C inhibition may be useful in quantitating numbers of repair complexes or repair enzymes active in a cell at a given time. For any 3-h period up to about 18 h, normal human cells show a constant rate of repair. After that time, repair drops off. With a more complete understanding of the nature of the ara-C effect, we can better understand the apparent alteration seen in the XP variant cells.

In many cases, however, chemical or physical agents damage the DNA of the cell either directly or indirectly in such a manner that the cell responds with an excision repair mechanism to remove the particular adduct produced by the agent in the DNA. The 5-bromodeoxyuridine photolysis assay is used to quantitate unscheduled DNA synthesis and to determine patch size. DNA excision repair in mammalian cells has been observed to occur in at least two distinct forms: "short-patch" repair as induced by ionizing radiation, and "long-patch" repair as induced by UV irradiation. Typical patch sizes ranged from 60-100 nucleotides for UV repair, 30-70 nucleotides for a variety of known and suspected UV-mimetic chemicals, to less than 10 nucleotides for gamma radiation. We find that agents such as N-acetoxy-acetylaminofluorene, benzo[a]pyrene diol-epoxides, bromomethylbenz[a]anthracene and 7,12-dimethylbenz[a]antacene, are inducers of long-patch repair. The alkylating agents ethyl methanesulfanate, methyl methanesulfanate, ethylnitrosourea, ENU, and N-OH-1-napthylamine induce both long- and short-patch repair. At low doses, nonsaturation of repair is seen in both XP and normal human fibroblasts, but at higher doses only normal human fibroblasts show a UV-like repair with a patch size of between 40 and 50 nucleotides. Through the use of labeled alkylating agents, we have been able to determine the approximate numbers of various lesions produced in the DNA of cells treated at different doses of agent. It is apparent from these studies that the most likely lesion to be inducing long-patch repair is O-6 alkylguanine, which is generally accepted to be the major precarcinogenic lesion following alkylation. Since O-6 alkylations are known not to cause helical distortions, it seems unlikely that the altered structure of the modified DNA molecule itself determines ultimate patch size. Work is currently under way in our laboratory to further elucidate factors influencing patch size.

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GENETIC ANALYSIS OF DNA REPAIR IN MAN WITH CELL HYBRIDS

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The long range goal of this project is to investigate the expression and individual genetic components involved in DNA repair in man. Such an elucidation of the genetic basis of DNA repair is fundamental if we are to understand its pivotal role in carcinogenesis. Therefore, the primary objectives of this project are (i) to genetically dissect the DNA repair systems in man, (ii) to identify the number and kinds of genes required for DNA repair, (iii) to assign these genes to specific human chromosomes, and (iv) to identify and determine the chromosomal assignment(s) of the genetic defect in xeroderma pigmentosum, a human skin disease which demonstrates direct linkage between defective repair of damaged DNA, induction of mutations, and induction of cancers.

These studies are being pursued utilizing man X mouse somatic cell hybrids segregating human chromosomes and several very sensitive and informative assays for DNA repair. This system makes it possible to distinguish human and mouse repair components, to determine gene-chromosome assignments, and to dissect this complex polygenic system by isolating its component parts. The hybrids assayed so far for excision repair ability appear to group themselves into one of three categories: (i) those having a magnitude of repair similar to human cells, (ii) those having mouse-like repair, and (iii) hybrids intermediate between the two.

Segregation of the ability to repair UV damage in 41 primary hybrid clones has been tested for linkage with 30 enzyme markers representing genes known to be assigned to each of the 21 different human chromosomes. Concordant segregation was not observed between DNA repair and these other enzyme markers indicating that the assignment of a gene(s) required for the ability to repair UV-induced DNA damage can be excluded from all but three human chromosomes. Cytogenetic analysis of informative clones will indicate whether the gene(s) required for repair of UV-induced DNA damage are carried on 1, 2, or 3 chromosomes.

The potential importance of this project is that it will yield information on the genetic structure of the DNA repair mechanisms in man. This information is essential if we are to fully understand the functional relationships between DNA repair, mutagenesis, and carcinogenesis.

Since this is a new research project (initiated September 17, 1979), no publications have as yet resulted.

GENETIC BASIS OF MUTA/CARCINOGENESIS

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An extremely wide range of factors, both inherited and environmental, are involved in mutagenesis and carcinogenesis in man. In order to fully understand how environmental and genetic factors interface, the Mammalian Gene Mapping in Somatic Cells Group has developed a project designed to elucidate the genetic basis of carcinogenesis and mutagenesis. The primary objectives of this project are: (i) to elucidate the genes important for susceptibility to mutagens and carcinogens; (ii) to identify the cellular genes required for RNA tumor virus infectivity, replication, integration and induction; (iii) to determine the chromosomal assignment of these genes; and (iv) to investigate the linkage relationships of homologous genes in man and mouse and other species. The data derived from these studies are important since an elucidation of the genetic basis of these systems is essential for our ultimate understanding of how genetic and environmental factors interact in the process of mutagenesis and carcinogenesis. Comparative mapping data will be important for the extrapolation of biological and biomedical data from laboratory animals to man.

We are pursuing these studies utilizing somatic cell hybrids as our experimental system which allows for the genetic dissection of complex polygenic traits by isolating their component parts, the determination of gene-chromosomal assignments of homologous genes in man, mouse, and other species.

We have analyzed somatic cell hybrids for aryl hydrocarbon hydroxylase activity and induction, an enzyme system known to convert aromatic polycyclic hydrocarbons to their mutagenic and carcinogenic forms. Thus far we have identified a gene that controls inducibility of this enzyme system and have suggestive evidence that this gene is located on mouse chromosome 17, close to the genes that control the immune response in the mouse. We have analyzed hybrid cells for C-type RNA tumor virus infection and replication and have identified and mapped the gene that controls infection by these viruses. We are presently determining where this virus integrates in the host cell DNA following infection, a necessary step in viral transformation. We have identified and mapped five regions in the human and mouse genomes which have been conserved through 80 million years of evolution separating these two species. These regions are very important in the comparative genetic aspects of mutagenesis and carcinogenesis.

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MAMMALIAN BIOCHEMICAL GENETICS

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The principal aim is to evaluate hazards to humans of exposure to nuclear and chemical by-products of energy production. Of primary concern is that an increase in the frequency of mutations in somatic and germinal cells will cause an increased rate of malignancy in persons exposed and a reduced physical and mental fitness of individuals in succeeding generations. Generally, the quality and frequency of mutations in progeny of exposed animals are being compared with spontaneous mutations in unexposed populations.

Murine α-thalassemia, a radiation induced mutation, is homozygous lethal at the late blastocyst stage of development. The clinical hematology of heterozygotes is similar to that in man; these heterozygotes are the only known experimental models of human α-thalassemia. The deficient synthesis of hemoglobin in both adults and embryos is due to the radiation-induced loss of the α-globin and embryonic x-globin genes. In contrast to the chromosome loss induced by irradiation, most spontaneous mutations result from base substitutions such as have been found for three new α-globin phenotypes in mice.

Studies on the misincorporation of [^{3}H]isoleucine in the β chain of rabbit hemoglobin were done to help interpret data on the elevated frequency of misincorporation of isoleucine in some Marshallese exposed to radioactive fallout. The sequence of amino acids at the N-terminus is Val-His-Leu-Ser-Ser-Glu-Glu-Lys-Ser and the nucleotide sequence for this region is GUG-CAU-CUG-UCC-AGU-GAG-GAG-AAG-UCA. Only one codon for the first nine amino acids, AGU for serine at position 5, can be changed to one of the isoleucine codons AUU, AUC or AUA by a substitution of a single base. In treated rabbits, substitution of isoleucine was not restricted to residue 5, which suggests that the misincorporation can occur through non-genetic translational errors during protein synthesis as well as by base substitution mutations.

We have been validating mammalian mutagenesis assay systems that have the following advantages; the cells at risk are in their natural environment and the expression of the mutation occurs in vivo. Somatic stem cells (bone marrow) exposed to X rays are introduced into stem cell deficient W/W^v compatible recipients, where only a few clones contribute to the establishment of a functional hemopoietic system. One clone with a mutant hemoglobin has been identified in the first twenty recipients tested. The expression of the mutant hemoglobin persisted for more than a year. A mouse strain resistant to 6-thioguanine has been developed. Genetic and biochemical studies are being undertaken to determine the basis of the resistance. This strain will be used to validate an in vivo HGPRT$^{(-)}$ mutation assay system developed for murine colony forming stem cells.

Man's physical fitness is largely dependent on his ability to cope with his natural environment. Immunoglobulins are indispensable macromolecules in defense of microbial diseases. IgA secretory system is the protective barrier between the individual and his environment. The regulation of basal serum IgA levels is under both genetic and nongenetic

control. The IgA phenotype of the mother modulates the expression of the genetic potential of its offspring, which may influence their competitive efficiency. The cellular basis of this maternal effect is being explored.

A knowledge of the organization of the mammalian genome is indispensable to understanding the mechanisms by which mutagens/carcinogens alter normal gene expression. We have been exploiting naturally occurring genetic variation in inbred mice to define two genetic systems of unique value in examining the effects of mutagens/carcinogens on (i) hormone-mediated gene expression, and (ii) age-dependent changes in protein synthesis and assembly.

The mouse submaxillary gland contains several arginyl-endoproteases that are genetically linked. Expression of the gene cluster is coordinately controlled and requires the presence of the hormones thyroxine and testosterone. Each endoprotease exists in at least two genetic forms. This system should be sensitive in mutation studies because the chromosomal region involves 6-10 genes, gene expression can be hormonally manipulated, and the genes are mutable.

The eye lens crystallins represent three homologous families (α, β, γ) of proteins that demonstrate age-specific changes in synthesis and assembly. The lens is particularly sensitive to radiation and chemically induced damage. Inherited variations in the lens crystallins are being exploited to determine the chromosomal location(s) of the crystallin genes and will be useful in analyzing the mechanisms by which radiation and chemicals affect lens development and cataract formation.

Another important area of study is an investigation of the effect of clastogens on meiotic cells. The sister chromatid exchange (SCE) assay has become an important mutagen testing system although the biological significance of SCE is unknown. SCE bears striking resemblance to meiotic crossing-over. Therefore we have been using gene markers to analyze the effects of known clastogenic chemicals on meiotic recombination frequencies along three chromosomes of the mouse. The monitored regions represent about 100 recombinant units (15% of the genome).

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Genetics of Microorganisms and Drosophila

MICROBIAL MUTAGENESIS AND CELL DIVISION

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W. D. Crow

This group attempts to understand the mechanisms by which ionizing radiation and other agents interfere with cell division in bacteria. In addition, we are trying to use this information to develop very rapid and simple assays for mutagenic agents. Most of our experimental work is centered on cell division mutants of Escherichia coli. Much of our recent work has made use of the lon mutants. These mutants cannot produce septa after exposure to ionizing radiation. As a consequence, the cells grow into extremely long, multinucleate filaments. We have reported that a particulate fraction derived from wild-type E. coli or other bacteria can reverse the filamentation process and stimulate the formation of septa. We have recently established that the active material is in a fraction derived from the cytoplasmic membrane. This fraction also contains the cytochrome-based respiratory activity. Current experiments suggest that the septation-promoting activity may be directly related to the oxygen-removing ability of the fraction. Other workers have observed that the survival of irradiated lon mutants was improved if the medium in which the cells were incubated was kept anaerobic during the immediate postirradiation period. Our septation-promoting fraction is an excellent agent for removing the last traces of oxygen from the postirradiation growth medium. Destruction of the respiratory activity of the fraction by exposure to long wavelength ultraviolet light simultaneously destroys the septation-promoting activity. Cytoplasmic membrane fractions from anaerobically grown cells lack both respiratory and septation-promoting activity. In the future we hope to explain why lon mutants require a postirradiation anaerobic period in order to recover their ability to undergo normal division.

Although we do not yet have a complete understanding of the events responsible for the failure of septation in lon mutants, we are attempting to use them in simple, short-term assays for mutagenic agents. It is already known that several physical and chemical agents that react with DNA induce filamentation in lon mutants. The filaments develop in 1-3 h after exposure and may be 100 or more times the length of a normal cell. They are, therefore, readily observable by the use of a low-power phase microscope. We are currently attempting to determine the conditions that will distinguish known mutagens from nonmutagens. The most promising technique involves treating the bacteria with the test agent in nutrient broth suspensions. After 2-1/2 h of incubation, a sample is removed for microscopic observation. Using this approach, eight commonly used mutagens have been recognized by their ability to induce filamentation. Non- or weakly mutagenic agents such as phenol, saccharin, phosphate buffer, and physiological saline do not do so. We will expand the list of tested agents in order to determine the correlation between this assay and other assays based on microbial systems.

FURTHER STUDIES ON MUTATION INDUCTION IN HAEMOPHILUS INFLUENZAE BY N-METHYL-N'-NITRO-N-NITROSOGUANIDINE

R. F. Kimball

Evidence has been accumulated for a two-lesion hypothesis for mutation induction in Haemophilus influenzae by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). The two lesions are hypothesized to be an alkylated base leading at replication to a single-strand base substitution (SSBS) in the daughter strand and a noncoding lesion nearby in the opposite strand leading at replication to a gap in the daughter strand. Recombination repair of the gap will convert the SSBS to a double-strand base substitution, i.e., a completed mutation. Otherwise the SSBS will be eliminated shortly after replication by the mismatch repair system. The evidence for this hypothesis is circumstantial but highly suggestive.

Escherichia coli has been shown by Cairns and colleagues to have an error-free repair system for alkylation damage, including that induced by MNNG. The system is induced by exposure of the bacteria to low levels of the agent with the result that the effectiveness of subsequent high level exposures in inducing mutations is greatly decreased. The induction of the system is prevented by inhibition of protein synthesis with chloramphenicol. Neither pretreatment of H. influenzae with low levels of MNNG nor the presence of chloramphenicol had any effect on the amount of mutation induced by a subsequent high level exposure. Thus H. influenzae lacks the inducible error-free repair system found in E. coli. Both species have a constitutive repair system, but it is much slower than the combined induced and constitutive system. Some of the differences between E. coli and H. influenzae in their response to MNNG may be due to this difference in the rates of repair of the alkylation damage.

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STUDIES ON ICR-191-INDUCED FRAMESHIFT MUTATIONS IN HAEMOPHILUS INFLUENZAE

R. F. Kimball S. W. Perdue

Previous work on mutation induction in Haemophilus influenzae has employed agents that would be expected to produce mainly base substitution mutations. A detection system for presumed frameshift mutations from thymidine requirement to thymidine independence was developed using the well-known frameshift mutagen ICR-191. It was shown that this mutagen acted only on DNA that was in the process of replication during treatment. This contrasts with the earlier results with MNNG which showed that lesions introduced in various parts of the DNA could be converted to mutation when the replication fork reached them. The results with ICR-191 agree in this respect with earlier results by Newton and colleagues with Escherichia coli. No evidence for an effect of a recombination defect was found in either species when treatment was carried out during exponential growth. However, in H. influenzae, recombination occurring during transformation is itself mutagenic causing a distinctly higher than normal frequency of "spontaneous" mutations. These results agree with the hypothesis of Streisinger and colleagues that agents such as ICR-191 induce mutations by encouraging mispairing at strand discontinuities. The main site of strand discontinuities during exponential growth is the replication fork region, and ICR-191 is effective only there. The discontinuities produced by recombination during transformation can serve as a site for frameshift mutation even in the absence of an inducing agent.

GENETICS OF REPAIR OF RADIATION DAMAGE TO DNA IN BACTERIA

D. Billen C. T. Hadden

The goal of this project is to understand how environmental agents interact to damage the DNA of cells and how the cells cope with this damage. In particular we are concerned with the nature of the repair systems involved in restoration of damaged DNA and the cellular response to radiation or chemical damage as expressed in effects on DNA elongation, initiation, and recombination.

We are studying the relationship between DNA polymerase I-directed repair synthesis and single-strand breaks in toluene-treated cells exposed to X radiation or alkylating chemicals. The methods include a study of the role of endo- and exonucleases in providing primer ends for repair synthesis and the use of protective agents or radical scavengers to determine

their influence on the production of DNA polymerase I primer ends following X radiation. We have determined that about nine single-strand breaks/10^9 AMU of DNA are induced per krad of X rays. We are currently studying the dose responses for X ray induced single-strand breaks, X ray-induced DNA polymerase I-directed repair synthesis, the extent of ligation or sealing of single-strand breaks by toluene-treated cells, and X-ray-induced single-strand breaks in the presence of certain well-characterized radical scavengers such as KI or cysteamine.

About 40 to 50% of the single-strand breaks are repaired in toluene-treated Escherichia coli and are responsible for the excessive nick translation seen in the absence of DNA ligase activity in permeabilized, irradiated cells. We have recently shown that bromouracil-labeled DNA, upon exposure to far-UV, results in DNA polymerase I-directed repair synthesis in our toluene-treated cells. In cooperation with Dr. Bruce K. Duncan (Institute for Cancer Research, Philadelphia) we are assessing the role of uracil-DNA glycosylase (ung) in the induced repair synthesis by studying several mutants possessing various levels of residual enzyme activity. The bromouracil-DNA system may provide a well defined DNA lesion which in toluene-treated cells and under appropriate conditions may be repaired only by the DNA polymerase I-DNA ligase pathway.

We are continuing to study the effects of low doses of damaging agents on DNA metabolism in Bacillus subtilis by use of an assay system for postreplication repair and excision of pyrimidine dimers that allows quantitation of the cell's response to very low levels of irradiation (below 5% of the D_{37} dose for wild-type cells). We have shown that the ability to do postreplication repair is correlated with the ability to carry out transduction, and that a loss of transformability can occur without loss of postreplication repair.

By use of the transformation system of B. subtilis, it has been possible to demonstrate that DNA irradiated in vitro can be repaired by postreplication recombinational repair. This model system will be used to determine whether postreplication repair is significant in the repair of DNA damaged in vitro by ionizing radiation and chemical agents This question is significant because postreplication repair does not remove lesions from DNA but rather moves them from one chromosome to another. If potentially mutagenic lesions are conserved in a population of growing cells, the genetic consequences are much more significant than if the lesions either kill the cell immediately or are excised from the DNA before it is replicated. Current indications are that in most situations excision and postreplication repair go on, so that DNA replication before removal of damage is less likely to be lethal than if postreplication repair did not occur.

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YEAST MUTAGENESIS

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The Yeast Mutagenesis Group is concerned with understanding the genetic and cellular factors controlling induced mutagenesis and DNA repair in the yeast Saccharomyces cerevisiae. This simple eukaryotic microorganism is particularly suited for coordinated genetic and biochemical approaches to problems involving health effects of radiations and environmentally relevant chemicals (1). Our efforts have been concentrated along the following lines.

Mutagenic mechanisms of important or unusual agents. We have found that hydrazine induces forward mutations by a mechanism requiring post-treatment DNA replication, unlike ultraviolet light or ionizing radiation which generates mutations before replication. If replication is inhibited following hydrazine exposure, induced mutability is lost rapidly in both wild-type and excision repair-defective strains, suggesting the existence of a pre-replicative error-free repair of hydrazine-induced premutational DNA lesions, a process not dependent upon UV-induced pyrimidine dimer excision repair activity. The cytotoxic and mutagenic activities of the potent antitumor drug, cis-dichlorodiammine platinum (II), have been studied in haploid yeast strains (4). DNA repair deficiencies introduced by rad3, rad5, or rad6 genes lead to enhanced cytotoxicity of this metal complex, providing indirect evidence that the induced genetic damage is subject to DNA repair processes (4). Another class of genotoxic agents, the heavy metal ions, is being investigated in collaboration with C. S. Dudney (Health and Safety Research Division). Mutants with increased or decreased cadmium ion cytotoxicity have been isolated and are being subjected to intensive genetic analysis in order to estimate the number of controlling genetic loci involved. Cadmium, a suspected human carcinogen, is also mutagenic in yeast under certain conditions. Cadmium mutagenesis is being examined in well-defined mutants with altered cytotoxicity.

Development and validation of new and improved short-term eukaryotic mutagenicity tests. We have focused considerable attention upon the CAN1 locus, which carries structural information for the arginine-specific permease enzyme, a plasma-membrane protein responsible for active transport into the yeast cell of L-arginine and its analogue, e.g. the toxic L-canavanine. All spontaneous and mutagen-induced canavanine-resistant derivatives of the wild-type (canavanine-sensitive) strain carry recessive mutant alleles of this locus. Such can1 mutants are very efficiently induced by a wide variety of physical and chemical agents. The molecular basis for this high susceptibility to mutational change is not understood nor has the arginine permease been isolated and purified.

In order to classify chemicals active in the CAN1 system as to whether mutagenicity is independent, dependent, or partially dependent upon DNA replication, we have developed an improved assay in which mutagen-treated cells are permitted to grow and divide for varying periods of time on defined agar growth media, prior to agar overlay with canavanine. The effect of DNA repair deficient rad loci is being studied with this system. In collaboration with F. W. Larimer (Biology Division), chemical and radiation-induced revertibility of known frameshift mutations, his4-519, his4-38, and leu2-3, is currently under investigation. Since these alleles are suppressible by unlinked tRNA dominant SUF mutations, we are using this system to develop an assay to detect frameshift events at CAN1.

The molecular basis for induction and expression of forward mutations at CAN1 is also under study. Temperature-sensitive can1 mutants have been isolated with a method involving

high selection pressure. In collaboration with E. G. Bernstine (Biology Division), we are separating yeast membrane proteins from a canl (ochre nonsense) mutant using a high resolution two-dimensional (iso-electric focusing, SDS polyacrylamide electrophoresis) gel system, with the goal of identifying the CAN1 gene product, arginine permease. Since CAN1 has been cloned recently, eventually it should be possible to determine how much of the gene contains structural sequences for the permease, and whether or not any induced canl mutants represent alterations of nonstructural (i.e. intervening or signal) regions.

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DROSOPHILA CYTOLOGY AND GENETICS

R. F. Grell C. E. Generoso

Our studies are directed toward a fuller understanding of the meiotic system, whose processes form the basis of eukaryotic genetics. Events occurring at this time are responsible for the precise partitioning of the genetic material from the double or diploid amount found in immature germ cells to the unit or haploid amount found in the gamete. Failure to achieve a strict equality of distribution leads to aneuploidy, a condition which is generally deleterious and frequently lethal to the zygote. In human populations it accounts for ~ 50% of spontaneous abortions and a substantial proportion of birth defects.

Events responsible for aneuploidy in both humans and the Drosophila female probably share a common origin and occur most frequently during the first meiotic division. This critical period is characterized by three essential and interdependent chromosome activities, namely, pairing, recombination, and segregation, in that sequence. We have been studying the interrelationships among the three components of the system to better understand the defects which lead to faulty segregations and aneuploidy. A fruitful approach involves alteration of the normal system through perturbations induced by external agents such as heat, radiation, or chemicals or by internal genotypic changes, i.e., point mutations or chromosomal rearrangements. Of special interest has been the recovery of a temperature-sensitive recombination mutant which permits near-normal recombination at the permissive temperature but which drastically reduces recombination at the restrictive temperature. The reduction is correlated with high frequencies of non-disjunction leading to many aneuploid progeny and ~ 75% of lethal zygotes. The temperature sensitive period has been precisely defined and found to coincide both with the S phase and the time for enhancement of recombination by heat in the

normal genome. Further, our electron microscopic studies reveal that this period is characterized by the presence of the synaptonemal complex (SC), a tripartite structure which lies between synapsed homologues and serves as a marker of homologous pairing. To examine the possibility that the restrictive temperature acts to reduce recombination indirectly by affecting the pairing process, the mutant has been studied at the ultrastructural level. Serial sections of oocytes developing at the permissive and the restrictive temperatures demonstrate that the SC is unaltered by heat, a result most simply interpreted to mean that heat acts directly on a faulty gene product engaged in the recombination process during the sensitive period. The discovery that homologues are paired during DNA replication in Drosophila prompted a similar study of the mouse in collaboration with E. F. Oakberg. Utilizing EM-autoradiography an examination of the premeiotic interphase stage of the mouse spermatocyte has shown that the SC is present during DNA replication in this organism as well. These results call for a reexamination of current dogma which places homologous pairing and recombination at meiotic prophase, well separated from premeiotic interphase and S.

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BIOCHEMICAL AND DEVELOPMENTAL GENETICS OF DROSOPHILA

E. H. Grell

The broad objective is to determine how genes control the orderly growth and development of an organism. Mutant genes which cause a defect in a chemical or developmental pathway have often been successfully exploited to probe the nature of a normal pathway. The rationale of our effort is to select a mutant which affects the pathway of interest and then use a battery of genetic tests to learn as much as possible about that pathway. The advantage in using Drosophila is that it is possible to select gene mutations, deficiencies, duplications, reversions, enhancers, and suppressors which are the raw materials for the genetic analysis.

The segmentation pattern of Drosophila provides a model system for studying how genes control development. The two-winged flies like Drosophila evolved from insects with four wings and these in turn had as remote ancestors primitive arthropods with an appendage on each segment. The tendency in evolution has been to make each segment different from the others. In Drosophila the second thoracic segment bears a typical insect wing, the third segment has a small structure called the haltere, and the first segment has no wing or haltere but only a small humeral callus and a bit of neighboring tissue. Mutations of the bithorax cluster of genes change the second and third thoracic segments and the abdominal segments, but not the head or the first thoracic segment. Genes which affect the first thoracic segment are uncommon. The most familiar mutation which affects the humerus is one called Hexaptera. It caused a tendency for a wing-like structure to replace the humeral callus. It had low penetrance and a variable expression. Stocks labeled Hexaptera no longer show the phenotype. We have discovered another mutation (called Humeropedia) which causes a homeotic transformation of the humerus into a structure which appears to be the base of a

leg. It is a sex-linked dominant which also causes abnormal eye facets. It is somewhat like Hexaptera in that the humeral transformation has a low penetrance, but in combination with another mutation the penetrance is over 90%. In flies with a mild expression of the mutant phenotype, there is a duplication of the humeral callus. In effect, the flies have two shoulders on each side. In more extreme expression, the duplicated parts fuse and a structure which resembles a base of a leg protrudes at the shoulder.

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Development and Reproductive Physiology

REPRODUCTIVE PHYSIOLOGY

R. A. Wallace Ziva Misulovin

The aim of work in my laboratory is to understand the cellular and hormonal mechanisms that are responsible for oocyte growth and maturation in vertebrates. For most of these studies teleost and amphibian oocytes are used because they readily lend themselves to a combination of micrurgical, biochemical and cytological approaches.

We have recently completed a survey of many types of teleosts and found examples wherein recruitment phenomena occur at each of the various stages of oocyte growth, so that by a judicious choice of season and animal, we can now experimentally address the environmental, hormonal, and cellular processes which work in consort to recruit a clutch of a defined number of oocytes from any one of the various stages of oocyte growth into the next.

A major achievement in our laboratory has been the perfection of procedures for growing amphibian oocytes in vitro. Two key observations have been that rates of growth for cultured oocytes exceed those of oocytes in vivo and that "full-grown" oocytes continue to grow again when removed from the ovary and placed in our hormone-free culture system. This indicates that the normal growth of the oocyte is constrained by extrinsic, hormonally controlled processes within the ovary rather than by intrinsic limitations. The most important ingredient of the culture medium is vitellogenin, the hepatically synthesized precursor to yolk proteins. Oocytes from both Xenopus laevis and Rana pipiens were grown successfully over 2- to 4-week periods using X. laevis vitellogenin; the most interesting observation from these experiments was that oocytes from R. pipiens grew twice as fast as those from X. laevis, indicating that the maximum rate of growth is intrinsic to the oocyte and that the source of vitellogenin is irrelevant. The latter conclusion was confirmed by isolating vitellogenins from a variety of animals ranging from fish and amphibians to turtles and birds and all were found to be selectively incorporated by oocytes from either X. laevis or R. pipiens. These results suggest that vitellogenin from whatever source can support amphibian oocyte growth.

Finally, X. laevis vitellogenin has been resolved into at least four different peptides of similar molecular weight (182,000 to 197,000) but with slightly different amino acid

sequences. Once incorporated into the oocyte, vitellogenin is proteolytically cleaved into the yolk peptides lipovitellin-1, lipovitellin-2, and phosvitin. High resolution gel electrophoresis has resolved each of these yolk peptides into a family of at least three peptides. These results complement recent evidence obtained by gene cloning that X. laevis has four closely related vitellogenin genes.

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REPRODUCTION, EMBRYOGENESIS, AND TERATOGENESIS

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The aims of this work are to gain a better understanding of the events occurring during reproduction, development, and maturation in fish and amphibia and to use the methods and information that are developed to determine and evaluate the potential toxicity and teratogenicity of a variety of materials (mixtures and pure compounds) that are products of fossil energy processes.

A major event of oogenesis is the selective accumulation and storage of large amounts of yolk - a process involving cell membrane receptor sites. Despite the specificity of this process, non-yolk materials become incorporated and stored, thus presenting a potential toxic and teratogenic threat to the developing embryos. The hormonal aspects of oogenesis, ovulation, and maturation are also under study. In addition, cytological studies on the process of fertilization and early embryogenesis, using scanning and transmission electron microscopy, have elucidated events that occur prior to, during, and after gamete fusion. All of these processes are directly or indirectly affected by environmental factors. The information obtained from these studies will contribute to our understanding of the basic processes and also of how environmental parameters affect reproductive potential and capacity.

For studies of teratogenesis, embryos are exposed for varying periods (12 h to 5 day) and at various developmental stages to a range of concentrations of potential teratogens. Most substances tested appear more toxic to early embryonic (blastula/gastrula) and later larval stages than to intermediate (tailbud) stages. During each experiment, data are collected on LC_{50} and LC_{100} concentrations and on growth rates, pigmentation, and swimming behavior of the embryos. In all cases, though to varying degrees, all parameters are adversely affected by the toxicants tested suggesting that in a normal competitive environment the embryos would have difficulty surviving. Scanning and transmission microscopy is used to assess cytological damage. For the toxicants tested to date, severe muscle and nerve damage have been noted. This damage can be directly related to toxicant concentration, length of exposure, and developmental stage of the embryo.

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CYTOTOXICITY OF FOSSIL FUEL BY-PRODUCTS

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With the ever-increasing number of potentially environmentally hazardous chemicals being produced, there is an urgent need to develop methods for their rapid and inexpensive screening. For this purpose we have used a ciliate, Tetrahymena pyriformis. A variety of fossil fuel by-products and pure compounds have been tested. These include a large series of aromatic amines; and shale oil, coal liquefaction, and gasification by-products. Leachates from sludge and ash samples have also been examined. The parameters measured include population growth (reproduction), respiration, behavioral and shape changes in individuals, and cytological damage. Reproduction of the population is measured spectrophotometrically and reveals that at lower concentrations of toxicant the lag-phase of growth is generally extended. At higher concentrations, the final population density is reduced. Similar reductions in respiratory activity are also seen, and these are reflected in ultrastructural damage to the mitochondria. Other ultrastructural changes that have been recorded are disruption of the pellicle and cell membrane, discharge of mucocysts, and changes in the vacuolar system. Behavioral (swimming) and general morphological changes are also apparent and often characteristic for a specific type of toxicant. An important aspect of these studies is an attempt to relate the molecular structure (and other physical characteristics) of the suspected toxicant molecule to its toxicity. We have been able, for example, to demonstrate a positive correlation between the toxicity of a given class of compounds and their partitioning coefficients, boiling points, and molecular weights. We hope to develop data such as these so that once contaminants or toxicants have been identified, predictions can be made regarding their potential as environmental toxicants and/or teratogens.

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Mammalian Genetics and Teratology Section

Section Overview

The work of the Mammalian Genetics and Teratology Section includes research in mutagenesis, basic genetics, reproductive biology, and teratogenesis. The findings reported here range from the very basic (e.g., detailed aspects of gene action) to the very applied (e.g., testing of complex mixtures), with many gradations in between. This overview will, for the purposes of summarizing, discuss them under three headings: basic studies, method development, including exploration of the biological material, and testing.

Basic Studies. The basic studies have made good use of the wealth of genetic material that has accumulated in mutagenesis experiments of various kinds (stocks carrying mutations at the specific loci or gross chromosome aberrations), or of the findings of mutagenesis experiments themselves. In the latter category is the finding of a repair system in the fertilized egg, a discovery that was made when chemical exposure of males from the same strain of mice yielded radically different dominant-lethal frequencies, depending on which strain of unexposed females was used in the mating. The relation of this end point to others, and the genetics of repair competency or deficiency are now under study.

A detailed genetic, embryological, and cytogenetic analysis of over 100 mutations involving one of the specific loci has resulted in the definition of 13 complementation groups in that region and has revealed a number of properties of induced and spontaneous mutations. Making use of this genetic material, as well as of several gross chromosomal aberrations involving the same chromosomal region, we have demonstrated a linear relationship between gene dosage (1x, 1.5x, 2x, 3x) and level of expression of an enzyme, mitochondrial malic enzyme (MME). In the course of this work we discovered a regulatory gene, active in only one tissue (brain), which, in cis position with the closely-linked structural locus, determines the rate of synthesis of MME. These discoveries have provided unprecedented opportunities for studying pathways of gene regulation in a mammal.

Other opportunities for the study of gene action are provided by a number of X-autosome translocations [T(X;A)'s] which continue to be discovered in the course of mutagenesis experiments. In these rearrangements, X-chromosome inactivation extends to neighboring autosomal loci. Autoradiographic studies with a number of these T(X,A)'s provided evidence on tissue-specific selection (due to inactivation of critical autosomal loci) as well as allowing certain conclusions about X-inactivation pathways.

The T(X;A)'s, all of which are male sterile, have also provided tools to study genetic prerequisites for male fertility. Making experimental chimeras between T(X;A) and normal males, we showed that the cause of sterility resides in the germ cells, rather than being organismic. There is also evidence, from attempts at genetic "rescue" with a duplication, that inactivation of autosomal loci is probably not responsible for the sterility. -- In cases of genetic sterility where the sex-chromosomes are not involved, there is almost always a translocation with breakpoint(s) near chromosomal ends.

Method Development. A large number of the major findings reported by the Section concern method development and exploration of the properties of the biological systems used. Considerable progress has been made in developing the skeletal mutation system, which provides

information on dominants that is highly useful for risk assessment (results have been used by national and international committees). Characterization of some of the mutations has shown, among other things, that recessive-lethal mutations can have complex dominant deleterious effects. A sensitive-indicator test is now under development which will make the screening for skeletal mutations much faster and easier.

Method development has also progressed on the in vivo somatic-mutation test ("mouse spot test"), which was originally designed by us and is now being widely used as an in vivo screen for mutagens. Another method developed here is the numerical sex-chromosome anomaly (NSA) test for nondisjunction, which now stands up well in comparison with a number of purely cytological screens for nondisjunction. The NSA method is being used by us to explore the effects of female age on chromosome loss and nondisjunction. Literature reports of induced nondisjunction frequencies based on purely cytological studies were checked by measuring dominant-lethal frequencies (the latter should include all monosomies and most trisomies, in addition to other aneuploidies). The reported frequencies turned out to be greatly exaggerated.

A model for estimating the misclassification error was experimentally established for the heritable translocation test. -- To determine the relationship between unscheduled DNA synthesis (UDS) in the testis and the repair of genetic damage, studies were carried out that involved pretreatment with certain agents prior to exposure to known mutagens. Additionally, the UDS response to chemicals that react by an SN1 mechanism has been compared with that following SN2-type chemicals.

A rapid, easy, and sensitive in vivo screening test for teratogenesis was developed. The end point is a set of homeotic shifts in the axial skeleton. By working with an inbred strain that normally crosses the pertinent developmental thresholds, and concentrating on the stage of maximum sensitivity, positive effects can be found at very low levels of teratogen, e.g., 5 R X rays.

An in vitro teratogenic prescreen being developed in our Section makes use of teratocarcinoma-derived cell lines which have developmental capabilities analogous to early embryos. With this system, many parameters, including differentiational ability, can be assayed entirely in vitro. Other teratogenesis tests in progress involve in vitro treatment of early embryos and subsequent transfer to host females.

An exciting finding during this reporting period has been the super-mutagenicity of ethylnitrosourea (ENU) which makes this compound useful as a model chemical mutagen for determining the effects of many factors that influence mutagenic response. In contrast to radiation, ENU is much more mutagenic in spermatogonial stem cells than in meiotic stages of either sex, or in postmeiotic stages of the male.

Germ-cell stage was found to be more important than type of mutagen in determining the nature of sterility in first-generation offspring. The question of germ-cell stage differences was also approached by the use of molecular dosimetry, utilizing radio-tracer techniques. It was found that protamine (which is present only in spermatozoa or late spermatids), as well as DNA, is an important molecular target in the induction of chromosome aberrations.

Oocytes received some attention in attempts to build bridges between mice and human beings. By use of a method developed by us for timing oocyte growth, it was demonstrated that the transition from mutationally insensitive to sensitive occurs at an oocyte stage that is similar in all mammals so far investigated. -- Mutation rate was determined for females irradiated at birth when several properties of mouse oocytes resemble those of humans. The

mutation rate was low, and, as in the case of adults, there was a marked dose-rate effect. The results also showed that there is no correlation between resistance to oocyte killing and sensitivity to mutation induction.

Testing. Finally, many of the tests that have been developed and/or perfected here were used in several testing programs. An extensive test battery was applied in a study of Diesel exhaust fumes, with negative results, but a clear, though small, effect on female reproduction. A replicate of an earlier plutonium experiment was initiated, both specific-locus mutations and heritable translocations being determined. Pu deposits in the basement membrane of the testis and may thus provide continuous exposure of spermatogonia. A number of coal-related compounds and process mixtures from experimental synfuel technologies were tested by several of our standard methods. Ethylene oxide is the first industrially important compound tested that produces a significant increase in heritable translocations. A number of compounds on FDA's GRAS (generally recognized as safe) list are being tested by the UDS method. Inhalation of ethylene dibromide, a gasoline additive, was shown to produce alkylations in germ cells.

The participation of several members of the Section in national and international committees concerned with the problem of environmental mutagens has stimulated us to an in-depth examination of methods suitable for detection of mutagenicity and/or for estimating levels of various types of genetic risk to human populations.

Mutagenesis

PROBABLE DOSE-RATE EFFECT FOR INDUCTION OF SPECIFIC-LOCUS MUTATIONS IN OOCYTES OF MICE IRRADIATED SHORTLY BEFORE BIRTH

P. B. Selby S. S. Lee
E. M. Kelly

Oocytes present near the time of birth in mice may be the most relevant stage in the mouse for estimating genetic risk in women because of their considerable resistance to being killed by radiation and their condensed chromosome structure. At the least, they represent one of three major groupings of mouse oocytes that can be easily studied. A few years ago we found that the specific-locus mutation frequency following acute exposure to 300 R of X radiation within 9 h after birth is about one-third as high as that of comparably exposed spermatogonia of adult mice. We just finished an experiment designed to test whether there is a dose-rate effect in these oocytes. The dose rates used were 0.8 R/min (gamma radiation) and 93 R/min (X radiation), with the total exposure of 300 R being given just before birth. The frequency in the chronic experiment (1 mutation in 37,218 offspring) is significantly lower than that in the combination of the concurrent acute experiment (3 in 16,194) and the acute experiment done earlier (3 in 14,259), $\alpha = 0.036$.

These results are consistent with there being a marked dose-rate effect. Whatever the explanation, it is clear that these germ cells have an exceptionally low mutational response. The data also show that there is not a positive correlation between radioresistance to cell killing and radiosensitivity to mutation induction.

RADIATION-INDUCED DOMINANT LETHALITY IN FEMALE MICE

P. B. Selby G. D. Raymer
S. S. Lee

Frequencies of induced dominant lethality in mice, together with estimates in humans of the fraction of unbalanced conceptuses that could survive birth to be handicapped, provide a means of constructing an absurd overestimate of the genetic hazard from radiation-induced unbalanced gametes. Such an overestimate is useful in risk estimation because it provides a way of judging the reasonableness of more commonly used ways of estimating genetic hazard from induced chromosomal disorders, especially if the overestimate is lower than the conventional one. Because of claims in the literature (for example, Uchida and Lee, Nature 250:601-602, 1974) that radiation induces considerable nondisjunction in female mice, and because of the relative sparcity of data for use in estimating genetic risk for gross chromosomal effects in the female, we have been capitalizing on this new approach based on dominant lethality in an attempt to improve genetic risk estimates for women.

From the mass of data that has been collected and is being analyzed, it already seems clear that low-dose-rate exposure (about 310 R spread over 6 weeks) results in a considerable decrease in the frequency of dominant lethality as compared to acute exposure, as was expected. These and other of our results disagree radically with the yields of dominant lethality that would be predicted from Uchida and Lee's conclusions. Our results seem consistent with very low genetic hazard from gross chromosomal damage following low-level exposure of oocytes.

INDUCTION AND NATURE OF DOMINANT SKELETAL MUTATIONS

P. B. Selby S. S. Lee

Dominant skeletal mutations are being studied in an attempt to assess total induced genetic damage to one entire body system. Our earlier results have formed the basis for a major new approach (adopted in the 1977 UNSCEAR Report and the upcoming BEIR III Report) for the estimation of genetic hazard from radiation in the first generation. Our recent research has been in two areas: (i) genetic analyses to reveal some aspects of the nature of the mutations with which we are dealing, and (ii) the development of the sensitive-indicator method, a much more rapid approach for estimating a large part of the induced dominant damage to the mouse skeleton.

For three of the dominant skeletal mutations, we have shown that the affected mice are carriers of balanced reciprocal translocations. This was demonstrated by showing that for each there was a perfect correlation between the presence of the dominant mutation and the translocation (demonstrated by presence of multivalents in diakinesis-metaphase I cells in the male), and by taking into account the frequencies of induction of heritable translocations and of dominant skeletal mutations. This finding shows that effects in balanced translocation carriers must not be ignored in estimating genetic risk, as they were

before these results became known. Cytogenetic and genetic analyses are in progress to locate the breakpoints for these three translocations.

Another eight dominant skeletal mutations were tested for effects in homozygotes. All have been shown to be recessive lethals, seven of them killing between implantation and birth, and the eighth killing usually (or always?) after 2 weeks of age. Animals homozygous for this mutation have retarded growth and more severe skeletal malformations than are found in heterozygotes.

Interestingly, two of the eight homozygous lethal mutations have incomplete penetrance for low levels of dominant lethality. A few others are still being tested in this regard. The results show clearly that many dominant skeletal mutations are homozygous lethal, and conversely (and at variance with some claims in the literature) that recessive lethals often have dominant deleterious effects.

The sensitive-indicator (SI) method is based on our earlier finding that a few specific, easily observed, anomalies are included in the syndromes produced by a sizable fraction of all dominant skeletal mutations. Coded skeletons of progeny sired by treated or control males are examined for a few specific malformations, these being the SI's. Other presumed dominant skeletal mutations, based on "presumed-mutation criteria" (Selby and Selby, Mutat. Res. 50:341-351, 1978), are also recorded. Various radiation and chemical treatments of spermatogonia are being used to determine the efficiency of this method and whether the relative mutation frequencies for dominant skeletal mutations under different conditions parallel those found using the specific-locus method.

To date, a total of 2209 skeletons have been examined, almost exactly one-half of which are from the untreated control. Of the 19 presumed mutations, 17 have come from the treated groups. None of the 11 SI's has been in the control. Presumed mutation frequencies are significantly higher than the control for the following three treatments so far: 600 R acute X radiation, 100 R + (24 h) + 500 R acute X radiation, and 150 mg/kg ethylnitrosourea. Confidence limits for ethylnitrosourea are still broad, but the data are consistent with its being a supermutagen for dominants, as it is for specific-locus mutations. The SI method shows great promise, and the results of the experiments in progress will be of considerable importance in risk estimation for radiation and chemicals.

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GENETIC EFFECTS OF PLUTONIUM IN MICE

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The primary purpose of this program is to determine, both qualitatively and quantitatively the potential hazard from genetic effects of plutonium. The approach focuses on mutational damage in mice that is actually transmitted to descendant generations. Both of the two main types of genetic effect are measured: (i) gene mutations and small deficiencies are scored by the specific-locus method developed at this laboratory, and (ii) major chromosomal aberrations are measured by the heritable-translocation test. The program was started because no work had been done on transmitted gene mutations induced by plutonium in the spermatogonial stem cells, the cells of primary importance in man under the conditions of human exposure.

Because the first experiment with injected ^{239}Pu citrate in this program gave a lower mutagenic effect than expected, an equal-sized replicate is being conducted in order to shrink the rather wide confidence intervals on the point estimates of the mutation frequencies for both end points. This should also help to determine whether or not the mutational damage increases over extended periods of time, as would be expected from the assumption of continuous uniform exposure of the spermatogonia.

The benefit to be derived from this program is that, whether or not plutonium turns out to be a sizable genetic hazard in man, it is essential that the Department of Energy be in a position to cite definitive results on transmitted genetic damage in mice to indicate qualitatively and quantitatively what the risks actually are. The information is needed as an integral part of environmental impact statements and of health-effect evaluations.

MOUSE SPECIFIC-LOCUS MUTATION EXPERIMENTS WITH DIETHYLNITROSAMINE AND ETHYLNITROSOUREA

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The mouse specific-locus test, developed at this laboratory 33 years ago, is still the only feasible and reliable method for measuring the rate of transmitted gene mutations and small deficiencies induced by radiation or chemicals in mice. It is, therefore, of vital importance both in risk estimation and in developing a basic understanding of chemical mutagenesis in mammals.

Diethylnitrosoamine (DEN), chosen for study because of its extremely potent mutagenic effect in Drosophila, proved non-mutagenic in the mouse. (Only 3 mutations were obtained in 60,179 offspring, where 3.2 mutations would have been expected on the basis of historical controls.) In this case, a mutagenicity test on an organism as high in the tier system as Drosophila is clearly not a reliable guide of what to expect in mice. DEN requires enzymic activation, and it is possible that the mouse testis is neither activating it nor receiving the mutagenic metabolite from activation elsewhere in the body. This was investigated by a mouse specific-locus test with ethylnitrosourea (ENU), which is also highly mutagenic in Drosophila but requires no activation.

ENU has turned out to be a supermutagen in the mouse. It is 20 times more mutagenic than procarbazine, heretofore the most mutagenic chemical known in the mouse. It is sobering to realize that a single injection of ENU gives a mutation rate 73,000 times higher than would result from the total dose of ionizing radiation accumulated in 1 year at the level of maximum permissible human exposure. Because of its high mutagenicity, ENU is serving as a useful model chemical mutagen for determining the effects of the whole array of factors that influence mutagenic action. The dose-response curve, the responses in the two sexes, the effects in different germ-cell stages, the qualitative nature of the mutations, and the influence of other biological factors on ENU mutagenic action have already been explored.

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USE OF THE SPOT TEST AND THE SPECIFIC-LOCUS TEST TO SCREEN FOR MUTAGENS ASSOCIATED WITH COAL-RELATED TECHNOLOGIES AND TO QUANTIFY THE HAZARD

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M. G. Hayes

The EPA Pass-through program has funded work to screen for mutagens associated with coal-related energy technologies and to quantify gene mutation hazards from such mutagens. The former portion of this effort is accomplished primarily through the in vivo somatic-mutation test ("mouse spot test"), and the latter through specific-locus experiments.

Within the same general project, work has also been carried out to validate the spot-test method (which was originally developed by us over 20 years ago, using radiation) and to obtain some further clues about the nature of the mutational events scored as spots; this work has involved a number of chemicals that are not coal related. During the reporting period, for example, we attempted to determine whether a probable repair inhibitor could be used as a potentiator of mutagenesis. Caffeine was administered in various ways, with or without radiation, and radiation was given with or without caffeine. We found additivity of effects, instead of potentiation or inhibition. In looking for a chemical that fulfills the requirement for a suitable spot-test control, we investigated ethylnitrosourea (ENU) and found that, of a considerable number of chemicals investigated by us, ENU (at 50 mg/kg) yielded by far the highest mutation rate ever found in the spot test.

Coal-related chemicals and mixtures that have been studied by means of the somatic-mutation (spot test) screen include benzo[a]pyrene, and various products of experimental synfuel technologies, such as Coal Liquid A = Synfuel A-3 (both, the crude and the neutral fraction) and Coal Liquid No. 2 (SRC process). Using the specific-locus test for heritable point mutations, the following coal-related compound and mixtures have been (or are being) investigated: SO_2 (as sodium bisulfite), benzo[a]pyrene, dimethylbenzanthracene, Coal Liquid A, and Coal Liquid No. 2.

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TESTS FOR HERITABLE EFFECTS INDUCED BY DIESEL EXHAUST IN THE MOUSE

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The effectiveness of inhaled whole diesel exhaust in inducing heritable effects in the mouse was studied by the following battery of tests: specific-locus test in males, dominant lethals in males, heritable-translocation test in males, dominant lethals in females, and total reproductive capacity in females. These assays were chosen to detect a number of genetic end points, namely, point mutations, chromosome breakage, and chromosome interchange. Effects were looked for in a variety of germ-cell stages. Ancillary studies involved germ-cell survival in the male and reproductive performance in the female.

Mice were shipped to the EPA laboratory at Cincinnati for exposure to diesel exhaust from a Nissan engine, which delivered 6 mg of particulates/m^3 within the exposure chambers in which the mice were housed 8 h per day, 7 days per week. Controls were housed in equivalent chambers receiving a flow of air. Exposures lasted for 5 to 10 weeks, depending on the end point studied.

The results of all genetic tests in both sexes were negative. Small but unequivocal effects on the reproductive performance of females could be observed, consisting of a decrease in the number of ovulations and an increase in the interval between mating opportunity and copulation. It is not known whether these effects were the result of direct damage to the ovary or to some other endocrine organ (e.g., pituitary). The absence of genetic effects could thus indicate either that no active metabolites reached the gonads or that the germ cells are refractory to induction of mutational events by such metabolites. Regardless of the explanation, it appears that transmitted genetic effects are not a major hazard from exposure to diesel exhaust.

IN-DEPTH EXAMINATION OF MOUSE MUTAGENICITY METHODS

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A number of national and international committees are presently concerned with the problem of environmental mutagens. At least three major committees have been at work in trying to identify (i) suitable test batteries for the detection of mutagenicity, and (ii) methods for estimating the level of various types of genetic risk to human populations. Whole-mammal mutagenicity tests play a vital role in developing both of these objectives. We have been heavily involved in the efforts of the following deliberating bodies: International Commission for Protection against Environmental Mutagens and Carcinogens, GENE-TOX, and the International Agency for Research on Cancer. This has resulted in our producing a number of extensive documents that review whole-mammal mutagenicity tests, examine the advantages and drawbacks of each, develop quantitative criteria for negative as well as positive results, and recommend protocols.

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HERITABLE TRANSLOCATION TEST IN MICE

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The mammalian testis is known for its high sensitivity to chromosome breakage effects of ionizing radiation and of certain chemical mutagens. Consequently, this type of genetic damage is of serious concern in the evaluation of genetic hazards posed by radiation and by chemicals in the environment. From this standpoint the heritable translocation test (HTT) in mice is generally regarded as an important method, both for detecting chromosome breakage effects and for assessing genetic risk to humans. We are currently working on the improvement of the HTT for use in practical testing. At the same time we use this method in studying the effects of various conditions of radiation exposure on the induction of chromosomal aberrations in spermatogonial stem cells, and in the practical testing of complex mixtures from coal conversion technologies and of specific compounds that are suspected to be of genetic hazard to exposed humans.

The HTT still needs improvement. One of the most important difficulties in evaluating the validity and significance of negative results has been the lack of an accurate measure of the error in classifying an animal normal when it is, in fact, a translocation heterozygote. We have completed a study which provides a model for estimating the misclassification error.

We have been studying the effect of X-ray dose and dose fractionation on the induction of heritable translocations in spermatogonial stem cells. In our study on dose effects (doses of 150, 300, 600, and 1200 R) we found that the frequencies of reciprocal translocations at all doses were significantly higher than the spontaneous level. The dose-effect curve was clearly "humped," with the highest frequency at 600 R, significantly higher than rates observed at 300 or 1200 R. The frequency observed at 300 R was significantly higher than that at 150 R. Our study on the effect of dose fractionation is still under way.

We regularly employ the HTT in practical testing and have recently studied two compounds that are produced on an industrial scale, find many uses involving wide-scale human exposure, and are known to be effective mutagens in various submammalian species. One of these, ethylene chlorohydrin, produced no significant increase in heritable translocations. On the other hand, significant increases were found with ethylene oxide. At one dose, the frequency

was 9.4% (38/406, compared with 0/1514 for the control). Ethylene oxide is the first industrially important compound that has been shown to induce heritable translocations in mice.

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REPAIR PROCESSES AND MUTATION INDUCTION IN MOUSE GERM CELLS

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This program of research attempts to study in a comprehensive manner the role of repair processes in the egg on the formation of chemically induced chromosomal aberrations in male germ cells of mice. Objectives are: (i) to understand the basic mechanisms by which premutational lesions produced by chemicals in male germ cells are converted into aberrations; (ii) to characterize stocks of mice and identify those that are best suited, from a repair standpoint, for practical screening; and (iii) to obtain information on the genetics of repair processes in the mouse.

The relationship between repair processes and induction of mutations in mammalian germ cells has not been studied to any significant extent until recently when we discovered the existence of a genetically controlled repair system in the fertilized egg of mice. To demonstrate the existence and magnitude of repair in fertilized eggs and to separate the effects of this process from other factors, we used a simple procedure whereby males from one stock, (101 x C3H)F_1, were treated with a mutagen and then mated to untreated females from four stocks [random-bred T-stock, (C3H x C57BL)F_1, (C3H x 101)F_1, and (SEC x C57BL)F_1] at selected periods after treatment. In all cases, the times when males were mated corresponded to postmeiotic germ-cell stages. Afterwards, the yield of dominant-lethal mutations in each of the four stocks of females was determined. The maximum difference between strains of females in the yield of dominant-lethal mutations found for isopropyl methanesulfonate (IMS) was an order of magnitude between the extremes. Small differences were found for ethyl methanesulfonate (EMS) and triethylene melamine, and no difference could be demonstrated for X rays. The differences observed between stocks of females strongly suggest that there are differences in the capabilities of the egg to repair the premutational lesions that were induced in the male genome. In the case of IMS, cytological study of first-cleavage metaphases showed that the relative incidence of chromosome aberrations clearly paralleled that of dominant-lethal mutations, thus confirming the repair-capability hypothesis.

Studies were also undertaken to determine if the repair processes in the fertilized egg affect the production of heritable translocations by EMS and EMS. Until this study was performed, a close relationship between the rates in which dominant-lethal mutations and heritable translocations are induced by mutagens (including EMS) had been the dogma. To our surprise, we found that this relationship does not hold for EMS. In the case of EMS we found that the heritable translocation data are consistent with the earlier dominant-lethal data in that higher frequencies of translocations were observed among the offspring of T-stock than of (C3H x 101)F_1 females. This result suggests that the process of exchange in chromosomes of EMS-treated spermatozoa and spermatids occurs after the sperm has entered the egg.

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DNA REPAIR STUDIES IN MAMMALIAN GERM CELLS

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It has been well established by us that unscheduled DNA synthesis (UDS), taken to be DNA repair, occurs in meiotic and some postmeiotic germ-cell stages of male mice exposed to a number of mutagenic agents. To measure this UDS, males are treated with a test chemical and are also given testicular injections of [^{3}H]dT. Sperm moving through the reproductive tract are recovered at various times after treatment and assayed for the unscheduled presence of [^{3}H]dT.

Chemical agents that are known to react by different mechanisms are being compared to determine if the amount of DNA repair induced in the germ cells is related to the type of reaction mechanism. Methyl nitrosourea, which reacts by an "SN1" type mechanism, has been found to induce 1.4 times more UDS in mouse germ cells than does methyl methanesulfonate (MMS), which reacts by an SN2 type mechanism.

Recent studies by us have shown that X-ray pretreatment of males reduces the UDS response of the germ cells to chemical mutagens, such as MMS, while pretreatment with caffeine enhances the UDS response to MMS. Such observations may be useful in determining the relationship between UDS and repair of genetic damage in mammalian germ cells.

Currently, a number of compounds on the Food and Drug Administration's GRAS (generally recognized as safe) list are being studied for their ability to elicit a UDS response in mouse germ cells. The chemicals are being administered both by i.p. injection and by gavage to determine how much the route of exposure may influence the UDS response. Studies with known mutagens have shown that the UDS response is somewhat lower with gavage treatment than with i.p. injections, possibly because a portion of the total chemical dose is being absorbed by the contents of the stomach and gut.

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CHEMICAL DOSIMETRY STUDIES IN MAMMALIAN GERM CELLS

Gary A. Sega James G. Owens

A number of chemicals have been found to be mutagenic in mammalian germ cells. However, different germ cell stages do not respond equally to the induction of genetic damage. Some stages can be greatly affected while others show little or no sensitivity. Also, the germ-cell stages which are affected will vary, depending on the type of chemical being used. Our chemical dosimetry studies are aimed at increasing our understanding of why the mutational response varies in different germ-cell stages and why different chemicals produce different spectra of genetic effects in the germ cells.

We are using radio-tracer techniques to measure the amounts of chemical mutagens reaching different germ-cell stages, to determine whether the molecular targets in the various stages are different and to see if DNA repair may be occurring (as measured by loss of DNA alkylation products with time). We have found that protamine (a small basic protein intimately associated with DNA and found only in late spermatid and mature sperm stages), as well as DNA, is an important molecular target for chemical attack that may lead to chromosome aberrations. Particular alkylation sites within germ-cell DNA and protamine are now under study.

Besides the usual i.p. injections of the chemicals, inhalation studies of selected, energy-related compounds are also being carried out to better reproduce conditions of human exposure. A recent inhalation study using ethylene dibromide, a gasoline additive, showed that an exposure level of 1 ppm-h (much below the permissible human exposure level) resulted in readily measurable alkylation of mouse germ cells.

Basic Genetics

GENETIC ANALYSIS OF MUTATIONS DERIVED FROM SPECIFIC-LOCUS EXPERIMENTS

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Specific-locus tests, which have for years been in use in our laboratory to measure the rate of induction of point mutations, yield mutations that can be recovered as stocks available for further study. This wealth of genetic material, suitable for the analysis of defined regions of a mammalian genome, is unparalleled elsewhere in the world. In analyzing the mutations -- by genetic, cytological, biochemical, and developmental means -- our purpose

is to discover the basic nature of the lesions and to relate this spectrum to the circumstances of the mutagenic treatment. The information gained also contributes to our knowledge of the organization of the mammalian genome.

In the past, we analyzed over 100 radiation-induced mutations involving the d and/or se loci. More recently, our efforts have been concentrated on c-locus mutations. Of 119 such mutations from radiation experiments, 16 were mosaics, and there was no evidence for radiation induction of this genotype. The mosaics appeared to be due to spontaneous mutation in one strand of the gamete DNA or in a daughter chromosome derived from pronuclear DNA synthesis of the zygote. Only a small minority of spontaneous c-locus mutants was not mosaic.

In the total array of c-locus mutants, some were albino (c^a) and others were intermediate alleles (c^x); however, the homozygous lethal classes contained only the former type of mutants, designated c^{al} type. Deficiency mapping of over 30 c^{al}'s indicates that 20 and 2 of these lethals are deficiencies of 1-6 cM and 4-8 cM, respectively, while 10 of the lethals lacked none of the flanking markers. On the basis of complementation mapping and other criteria, these 3 groups of lethals are divisible into altogether 9 complementation groups. In addition there are 4 other non-lethal complementation groups (for a total of 13), with viable mutants (c^{av} or c^{xv}) constituting the great majority (68 of 107) of the total sample.

Investigation of the time of death of c-lethals indicates that homozygous mutants die preimplantation, at (or shortly after) implantation, or neonatally. In none of 33 lethal stocks did the homozygotes die between days 9 and 19 postconception. The c and Mod-2 loci, and the region between them, were found to be unnecessary for survival in utero.

Analysis of the relation between radiation treatment and type of c-locus mutants obtained shows that mutations derived from X- or γ-ray irradiated spermatogonia are mostly viables (66.7%); and that the proportion of "intermediates" (almost certainly non-deficiency mutants) among these viables is the same as that among spontaneous c-locus mutations. There is no significant effect of dose rate on the proportion of lethals in the low-LET group.

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GENE DOSAGE EFFECTS AND THE GENETIC CONTROL OF ENZYME SYNTHESIS

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Work in our group concerns the biochemical and genetic analysis of mutant mice derived from specific locus experiments and the elucidation of the mechanisms by which the synthesis of particular enzymes is genetically controlled in the mouse.

The variety of chromosomal aberrations available at Oak Ridge offers rich possibilities for studies of the mechanisms by which these abnormalities produce their harmful effects in humans. We are studying the effects of certain aberrations on the level of gene expression. In the case of Mod-2, the structural gene for mitochondrial malic enzyme (MME), data obtained from animals carrying deficiencies, a duplication, and those doubly heterozygous for these

aberrations have shown a linear relationship between gene dosage and the level of expression of the enzyme. Work is in progress on animals that may show dosage effects for MME and several other enzymes in females due to their translocation to an X chromosome that is subject to inactivation.

Biochemical studies are also in progress on the effect of abnormal numbers of globin genes, either α or β, due to duplication or deficiency, on the ratio of α- to β-chain synthesis and on the levels of α- and β-globin mRNAs. These systems are of special interest due to their relevance to hereditary human diseases. Preliminary results indicate that the level of expression of the globin genes is not a simple function of gene dosage. It is possible that globin will provide an important exception to the principle of linear gene dosage effects.

We have discovered and characterized a gene which controls the level of MME expression in brain. The locus we have studied, Mdr-1, maps in the same region of chromosome 7 as Mod-2, the structural gene for MME, whose expression it controls. We have described two alleles at Mdr-1, $Mdr\text{-}1^a$ and $Mdr\text{-}1^b$, which determine high and low MME levels, respectively. Biochemical studies have shown that Mdr-1 determines the rate of synthesis of MME so that $Mdr\text{-}1^a/Mdr\text{-}1^a$ strains have more MME molecules than do $Mdr\text{-}1^b/Mdr\text{-}1^b$ strains. We have obtained definitive evidence that Mdr-1 acts in cis by an approach that directly measured the rates of MME subunit synthesis (MME is a tetramer) in animals heterozygous at both Mdr-1 and Mod-2. Our current research aims are to elucidate the mechanism by which Mdr-1 controls the expression of Mod-2 and to isolate the Mod-2 gene. Preliminary results suggest that $Mdr\text{-}1^a$ homozygotes possess more translatable Mod-2 mRNA than do $Mdr\text{-}1^b$ homozygotes.

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GENETIC TESTS FOR NONDISJUNCTION IN THE MOUSE

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Whole-chromosome aneuploidy is an end point not commonly studied in mutagenesis but potentially important because of the risk from trisomies which produce adverse effects late in development or even postnatally. Such whole-chromosome aneuploidy might come about through any of several basically different mechanisms that result in meiotic nondisjunction (ND). We have in the past devised a genetic method (the numerical sex-chromosome anomaly method) that is based on the facts that ND involving the sex chromosomes produces mostly viable mice, and that such exceptional animals can be recognized by the use of appropriate markers. During this reporting period, we researched the literature to compare the method with at least five techniques utilizing cytological ND indicators. Some of these techniques were found to be unreliable, and most are laborious. The numerical sex-chromosome anomaly method, by contrast, requires only external examination for the detection of exceptional types. It is sensitive in its detection of induced ND since spontaneous X^mXPY's are

quite rare and X^nX^nY's even rarer. In addition, the method is useful in detecting breakage-derived chromosome losses induced in females, where the dominant-lethal test is not easily applicable.

Because of the possibility that advanced age could increase induced and/or spontaneous ND, we are conducting an experiment in which irradiated or nonirradiated females of various ages, up to 13 months, are bred in a mating scheme that allows one to detect and distinguish between maternal or paternal trisomy, as well as maternal or paternal chromosome loss. It is already clear that advancing age does not detectably augment the frequency of induced loss, nor does it lead to many, if any, additional cases of trisomy.

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THE USE OF X-AUTOSOME TRANSLOCATIONS TO STUDY X-INACTIVATION PATHWAYS

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We have for some time been studying a series of mouse X-autosome translocations [T(X;A)'s] which were discovered here and which provided some of the major evidence for developing the single-active-X-chromosome hypothesis for mammals. Such translocations are valuable tools in the study of gene action since many autosomal loci are suppressed in a portion of the cells of heterozygous females.

By means of autoradiographic study of an array of T(X;A)'s having different breakpoints, we have recently demonstrated that, in each case, only one translocation product is subject to allocyclic behavior, and that there is thus no memory system. This finding, in conjunction with earlier evidence that degree of inactivation of translocated autosomal loci is influenced by breakpoint position, is most easily interpreted by assuming a single inactivation center with polarity in both directions. Other interpretations require special assumptions.

In short-term cultures from adult kidney of five T(X;7)'s we have found that there is nonrandomness in X-chromosome allocycly, with X^n predominantly (but not exclusively) inactive. This nonrandomness is not observed in two T(X;4)'s, nor does it occur in some fetal tissues of the T(X;7)'s. We propose that a region of chromosome 7 contains genetic material critical for growth or maintenance of kidney cells, and that inactivation of this region in T(X;7)'s results in tissue-specific selection. Translocations in which X^n exclusively is inactive are presumably extreme examples of selection resulting from autosomal inactivation.

These studies on T(X;7)'s and T(X;4)'s have thus provided evidence of tissue-specific selection as well as allowing certain conclusions about X-inactivation pathways. In addition, we have initiated work on three newer T(X;A)'s that involve other autosomes, namely chromosomes 2, 12, or 17. Breakpoints are being mapped, both genetically and cytologically, and preliminary evidence has been obtained indicating that at least one of the new translocations exhibits nonrandom X inactivation.

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CHROMOSOMAL PREREQUISITES FOR MALE FERTILITY IN THE MOUSE

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In an attempt to improve our understanding of the overall picture of the genetic causes of male sterility and their relation to mutagen and/or to germ-cell stage treated, we are analyzing sterile males found in the F_1 generation of mice treated in various germ-cell stages with a variety of mutagens. Unlike the "partially sterile" F_1's, which have no problems with the production of germ cells, the sterile F_1 males all exhibit blockage in spermatogenesis or spermiogenesis.

We have studied 200 sterile sons derived from fathers treated with one of a considerable array of mutagens. These studies involve banding of chromosomes to determine breakpoint location, and histology of the testis to determine where, in spermatogenesis, blockage occurs. We have found that the type of mutagenic agent used is not as important as the stage treated in determining the nature of the sterility observed. Thus, sterile F_1 males resulting from the treatment of spermatogonial stages are rarely translocation carriers, while sterility in males derived from treatment of spermatocyte or spermatid stages is usually associated with reciprocal translocations. The breakpoints are generally located near the ends of the chromosome if the translocation involves two autosomes. If it is a Y-autosome or X-autosome translocation, breaks anywhere in either of the translocated chromosomes appear to result in sterility.

In another effort to elucidate the chromosomal prerequisites for full male fertility, we undertook attempts at genetic "rescue" of X-autosome-translocation [T(X;A)] males, which are invariably sterile. The sterility of these mice may possibly result from the circumstance that the changes undergone by the X during spermatogenesis serve to inactivate attached autosomal portions. Therefore we introduced one extra dose of some of these portions in the form of a duplication. No "rescue" has been found to date.

Aggregation chimeras were produced between T(X;A) males and normal males, using a complex set of markers for recognition. These chimeras were found to breed only from the normal portion of the testis, indicating that the sterility of T(X;A) males, instead of being due to organismic causes, is a property of the spermatogenic cells.

The analysis of silver-stained synaptonemal complexes is currently being employed in the study of chromosome behavior at pachytene stage in male carriers of different types of chromosome abnormalities.

Reproductive Biology and Teratogenesis

EFFECTS OF RADIATION AND CHEMICALS ON THE GONADS

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The precise manner in which surviving spermatogonial stem cells repopulate cell depletion induced by radiation and chemicals still is not fully understood. Combination of ^{3}H-thymidine labeling with radiation suggests that the cell cycle properties of spermatogonial stem cells are not altered by radiation. Further investigation of this problem will be made using colcemid and hydroxyurea to kill specific stages of the cell cycle. Also, radiation and chemicals will be compared in order to gain further insights on stem-cell behavior. The high selectivity of chemicals for induction of specific types of damage in specific cell stages is being used to examine the relationship of damage in the testis to transmissible effects. 6-Mercaptopurine is an excellent example for it is clear that chromatid and iso-chromatid breaks induced in A_4-In spermatogonia and scored at diakinesis-metaphase I do not lead to an increase in dominant lethality. Conversely, dominant lethals are induced in preleptotene spermatocytes, but these cells give no evidence of chromosome breakage in the meiotic divisions.

The application of the low induced mutation rates observed for early mouse oocytes to estimation of genetic hazards in the human female has been questioned both on the basis of different nuclear morphology of arrested oocytes in the two species and because early oocytes of the mouse are extremely sensitive to radiation-induced cell killing, whereas those of the human female are resistant. Our recent success in timing oocyte growth in the mouse demonstrates that the change in mutational sensitivity in the mouse occurs in a stage that is similar in both morphology and radiation response in all mammals so far investigated. It is clear that some mouse oocytes with chromosome morphology similar to that of human oocytes have low induced mutation rates. Therefore, differences between mouse and human arrested oocytes in degree of chromosome condensation and sensitivity to cell killing do not appear to be valid arguments against using the mouse data to estimate human risks.

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DEVELOPMENT OF A SENSITIVE, RAPID, IN VIVO PRESCREEN FOR TERATOGENESIS

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Because morphological malformations are perceived to be among the most harmful consequences of environmental interference in development, it seems advisable to include some morphological end points in the in vivo screening for chemical teratogens. In choosing end points for a potentially sensitive, yet simple, test, we have looked for naturally occurring variability within inbred (genetically uniform) strains. Such variability indicates that the underlying distribution on a scale of developmental potencies crosses a threshold, providing the most favorable genetic situation for detecting small induced shifts. The BALB/c strain was found to show variability with respect to degree of development of 14th rib, position of lumbo-sacral border, number of costo-sternal junctions, and number of sternebrae. Using, to start with, a relatively high dose of X rays (100 R), we established that there was a clear-cut period of maximum sensitivity on day 9¼ postconception. Irradiation at that time yielded, for each of the four characters, the maximum incidence of "posterior" shift.

Working at this stage of maximum sensitivity, we are attempting to explore the quantitative pattern of the homeotic shifts at increasingly lower levels of disturbance and to determine the frequencies of axial skeleton anomalies that correspond to each level of homeotic shifts, both with radiation and selected chemical teratogens, in order to determine whether the shifts by themselves could be used as predictors of certain levels of more severe effects. We have also explored the effects of various experimental variables and have succeeded in streamlining the procedures.

Radiation experiments indicate that, down to 25 R, easily demonstrable shifts occur in all four end points; and at 12.5, there are shifts in three of the four. Even at 5 R, there is a highly significant shift in the distribution with regard to development of the 14th rib; shifts in the other three characters have not been demonstrable so far.

In studying the effects of 50 or 100 mg/kg of benzo[a]pyrene on the same end points in the BALB/c strain, we found that, with respect to homeotic shifts as well as anomalies, these exposures were equivalent to X-ray doses between 25 and 50 R and between 50 and 100 R, respectively. Thus there appears to be no clear qualitative difference between the action of these two teratogens.

We conclude that exposure of the BALB/c strain at the critical stage in development and subsequent study of homeotic shifts provides a very sensitive and rapid method for the detection of environmental interference in developmental processes.

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TERATOGENIC MECHANISMS AND PRESCREEN SYSTEM

Ron Filler Linda Spurling

Our aims are to establish a sensitive and rapid prescreen for identifying putative teratogens and to examine various molecular parameters within the early embryonic developmental period in order to elucidate operative mechanisms involved in teratogenesis. Teratogenic pathways are thought to be initiated by differential cell-killing or slowing of cell-division rates, loss or alteration of the differential potential of primordial cell types, or combinations of these factors. Further, in man and other mammals a teratogenic agent may act not only directly on the embryo itself but indirectly by exerting an effect or the maternal organism.

Teratocarcinoma-derived cell lines, obtained from aryl hydrocarbon hydroxylase "responsive" and "non-responsive" mice, have developmental capabilities analogous to early embryos. These cell lines are assayed for their ability to activate and detoxify proteratogens, their susceptibility to teratogen-induced cytotoxicity, and their restriction of *in vitro* differentiational ability. At the same time, we determine the biochemical competency of mouse embryos directly to metabolize proteratogens. Subsequent developmental effects are assayed by transfering teratogen-treated embryos to pseudo-pregnant females.

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Cancer and Toxicology Section

Section Overview

The Cancer and Toxicology Section is concerned with the investigation of the mechanisms by which chemicals, radiation, and viruses cause the changes broadly identified as cancer. In addition, the study of mechanisms has been extended to include the nontumorigenic effects, such as short- and long-term toxicity, of various agents, especially those associated with fossil energy and fuels. In order both to assess quantitatively carcinogenic and toxic effects and to elucidate mechanisms, it is essential to develop appropriate molecular, cellular, tissue, and whole animal model systems and, therefore, we are involved in devising and validating new test systems. The application of the knowledge gained by the various studies is to help establish estimates of risk to the general population and to workers who are exposed to carcinogenic and other toxic agents. Furthermore, this knowledge can be applied to protection and prevention.

The art of *in vitro* and *in vivo* bioassays is almost becoming a science, and the list of substances that are mutagenic to cells and carcinogenic to animals increases at a rate even greater than inflation. But such information is of little worth unless we can derive an understanding in terms of risk to man. Therefore, the use of human cells and tissues and an examination of methods of extrapolation are becoming increasingly important activities. The highly successful work in this Division in radiation carcinogenesis over many years, which has always combined the quantitative aspects (such as the determination of dose response relationships) with studies of mechanisms, provides a guide, if not a template, of the direction in which studies with chemical agents must proceed.

The sectional and group structure of the Division does not reflect fully the intellectual bridges or the collaboration that span not only groups but sections. The strength of this type of interaction has made it possible for us to mount major new programs for the study of the health effects of synthetic fuels for the Department of Energy and to characterize and assess various compounds for the Army. The quality of these more applied research programs depends on the environment provided by the high quality of long-term studies in the Section and the Division.

The commitment to the mutual education of young students and ourselves is maintained, in particular, through our pre- and post-doctoral training programs in carcinogenesis research supported, and recently renewed, by the National Institutes of Health.

Fundamental research in cancer biology and biochemistry is the specific province of Molecular Carcinogenesis, although much of the work in other components of the Cancer and Toxicology Section, as well as other sections of the Division, could be defined in these terms. Indeed, several of the staff members whose research involves molecular aspects of carcinogenesis are primarily associated with other Sections and report their progress in that context. Research in molecular genetics of carcinogenesis focuses largely on the "transposon" properties of the genomes of retroviruses, as these genomes are both endogenous to vertebrate cells and capable of moving from one location to another in cellular DNA, as well as being directly associated with neoplastic transformation. The transposon structure of the DNA

genomes of endogenous murine N-tropic and B-tropic type C retroviruses is being elucidated, and their chromosomal location mapped in hamster-mouse cell hybrids. In a collaborative project with the Viral Carcinogenesis unit, the restriction by host Fv-1 genes of murine leukemia virus infection has been localized to the formation of supercoiled DNA duplexes by use of newly developed, highly sensitive methods for analysis of viral DNA intermediates; this restriction is mediated by a poly(A)-containing RNA product of the Fv-1 gene and appears to require protein synthesis as well. The capacity of retroviral RNA genomes to bind specific tRNA primer molecules is being explored as a means of detecting viral-like gene products in human cancer cells. Ribosomal RNAs of mouse, chicken, and human cells were also found to have this capacity, the human rRNA selectively binding 2 of 4 glutamic-tRNA isoacceptors, providing a potential clue to the elusive and still putative human tumor virus. A model of the mechanism of retrovirus induction by radiation and chemicals is being developed, and experiments have established that compounds such as hydroxyurea act as inducer. So far there does not appear to be a specificity to the alterations of cellular or viral DNA sequences associated with induction. Because of the increased understanding of retroviruses, the time seems ripe for investigating the role of endogenous retroviruses in the radiation induction of myeloid leukemia in the RFM mouse. In this strain only one ecotropic endogenous virus is induced or occurs naturally and the RFM mouse carries a locus associated with the specific restriction of this virus. There is the possibility that transposition of sequences of this endogenous virus may be linked to leukemogenesis.

Research in regulation of gene expression aims at defining in molecular terms the mechanisms determining expression of specific genes, how these are regulated by hormones, and the events responsible for dysfunction of gene expression in cancer. Recent progress includes: (i) recombinant DNA cloning of a cDNA specific for RNAs and DNAs cognate to the model enzyme tyrosine aminotransferase (TAT), a reagent essential to further analysis of its regulation; (ii) preliminary indications that both insulin and cAMP regulate TAT synthesis by effecting alterations in mRNA structure which improve its translational efficiency, but by different mechanisms; (iii) the demonstration that differentiation-associated changes in TAT expression can be accounted for by comparable changes in functional mRNA; (iv) the finding that the metallocarcinogen beryllium specifically inhibits steroid-mediated regulation of gene expression; and (v) development of an experimental system allowing analysis of the events responsible for rapid intracellular turnover of selected proteins. In corollary work, a library of cloned cDNAs specific for products of genes of special interest to regulation is being developed.

During the 1960's Biology Division biochemists developed a collaborative program with chemical engineers of the Chemical Technology Division, originating and developing reversed phase chromatographic (RPC) techniques for fractionation of nucleic acids. This Macromolecular Separations Program, initially focused on transfer RNA separations and providing purified tRNAs to scientists throughout the biomedical research community, has now turned attention to improvement of RPC as a means of isolating bacterial plasmids and restriction fragments of DNA. Initial successes hold the promise of significantly advancing the technology of the rapidly burgeoning area of recombinant DNA research. Newly developed techniques permit the isolation of supercoiled plasmid DNA directly from bacterial extracts; yield and purity are comparable to those from the conventional expensive and time-consuming centrifugal method, and the chromatographic procedure has the capacity of large scale application.

This group also operates a pilot plant facility designed for the cultivation of bacterial and other cells on a scale many times larger than that available to most biologists. The technology has been developed recently for the photosynthetic growth of the chemo-autotrophic organism Rhodospirillum rubrum and the enzyme ribulosebisphosphate carboxylase has been produced in quantity. Since 1978 collaborative work has been carried out with a number of university and NIH scientists, as well as with colleagues from Biology and other Divisions of ORNL.

The concern about chemical carcinogens, stimulated by groups of people with motives that are not always related to a desire for scientific endeavor, has spawned a belief that results from rapid and relatively cheap in vitro systems that detect mutagens can predict the future risk of cancer. The varied studies of several of our research groups are aimed at the tedious, but essential, defining of the metabolism of chemical compounds, the significant molecular lesions in macromolecules, especially DNA, their repair, and the effect of the lesions on mutation, differentiation, and malignant transformation at a cellular level.

A systematic examination of the biochemical differences between carcinogenic benzo[a]-pyrene and the noncarcinogenic isomer benzo[e]pyrene has led to the understanding of the difference in carcinogenicity. In the case of benzo[e]pyrene, the metabolism involves the K-region and not the Bay-region of the molecule. Such a result pinpoints the conformation important to carcinogenesis.

While structure-function analysis holds great promise for the identification of carcinogens, we will still have to rely on cellular and animal systems for quite some time. The use of the highly mutable P3 human cells co-cultivated with cells from different tissues, and thus varying in capability for metabolizing putative carcinogens, has been developed for the determination of the relative potency of carcinogens and organ specificity of various classes of compounds.

The fundamental facets of cell growth and differentiation are being probed by compounds that can induce terminal differentiation. It is somewhat of a puzzle that the popular phorbol ester promoters induce terminal differentiation in some cells and block it in others.

It has been found that normal cells in culture excrete nucleosides. The physiological mechanism regulating excretion is coordinated with the cell cycle in hamster embryo fibroblasts but not in a series of normal and transformed cell lines. The biochemical events that are regulated are UMP-ATP transphosphorylase, de novo synthesis of UMP, and RNA turnover. These phenomena and the associated technology are being used to study the chemical induction of malignant transformation in vitro.

Three groups have, for different reasons, selected skin as the in vivo model system for: (i) the determination of carcinogenicity, dose-response relationships, and relative potency of compounds and mixtures derived from energy and fuel production processes; (ii) the study of the morphological, biochemical, and immunological factors that may be important in susceptibility and the mechanisms for carcinogenesis (for example, the sebaceous glands, the vehicle used with the putative carcinogen, and the relative proliferative cell populations are all important), (iii) determination of the factors involved in ultraviolet radiation (UVR) carcinogenesis; and (iv) the determination of the mechanisms involved in the initial events of skin carcinogenesis and their expression or promotion.

One group has used tumor promoters as an experimental tool to investigate the changes involved in the initiation and expression of skin tumors. It has been found that the tumor promotion stage can be divided into two, and possibly three, stages which should allow a

dissection of the important events and their sequential nature. Inhibitors and stimulators of tumor initiation are being employed to investigate the critical event(s) in tumor initiation by PAH. There is a good correlation between the ability of agents to inhibit skin tumor initiation and their ability to counteract the covalent binding of the PAH to DNA. Since fluocinolone acetonide and a protease inhibitor (TPCK), which are potent inhibitors of promotion, also counteract the induction of dark cells, and since specific inhibitors of prostaglandin and polyamine synthesis are also potent inhibitors of tumor promotion, all of these factors may play a role in tumor promotion. There is evidence that the polyamines and prostaglandins are involved in a later stage of promotion. Epidermal cell culture systems from hamster and mouse are being developed in order to study epithelial cell transformation in vivo.

Another group is concerned with all the facets of estimating risk to humans from exposure to complex mixtures containing carcinogens. Thus studies have been required on: (i) the relationship of skin structure to tumor susceptibility, (ii) the DNA lesions induced by the compounds, in order to derive dosimetric information, and (iii) comparative studies on different strains using benzo[a]pyrene as a reference carcinogen, in order to provide a basis for estimates of comparative potency. Human skin either maintained in short-term culture or on athymic nude mice is being used to obtain information that will help in extrapolation from mouse to man.

Skin of hairless mice is used to investigate the mechanisms of UVR carcinogenesis. UVR is the major cause of the prevalent skin cancers in the United States, and studies are being carried out to determine if there is a correlation between specific UVR-induced lesions and subsequent cancer. If the molecular lesions can be quantitated in various species, then methods of extrapolation across species could be tested. In order to make assays of UVR-induced damage sufficiently sensitive, it has been necessary to develop suitable methods for measuring the DNA damage at fluences that are environmentally relevant. The versatility of skin as a model system for the study of carcinogenesis has encouraged our collaboration with Liane B Russell in the development of mouse stocks with a hairless phenotype and mutations that result in other variations in skin structure.

The studies of cancer development in the respiratory tract are of particular importance because they involve model systems that allow comparisons of in vivo and in vitro changes and because these studies are one of the few, but essential, investigations of in vitro transformation of epithelial cells. The combination of in vitro studies with tracheal transplants has made it possible to expand the investigation of the changes involved in neoplasms. Alteration (i) in morphology and cell populations, (ii) in growth characteristics, (iii) in differentiation, (iv) in the presence of various biochemical and immunological markers and (v) in the response to agents such as phorbol esters are all being elucidated.

A technique involving reseeding tracheas in which the epithelium was removed with tracheal epithelial cells has been developed whereby the growth of normal and preneoplastic cells can be studied. With this model system one can take advantage of both in vivo and in vitro techniques. An epithelial focus assay has also been developed whereby proliferative and neoplastic growth can be quantitated after exposure to carcinogens.

With the increasing emphasis on effects of inhaled environmental agents, especially from fossil fuels, our inhalation studies have expanded. It has been shown that exposures to cadmium aerosol causes changes in pulmonary function that are similar to changes in emphysema. Multiple exposures result in an adaptation by an unknown mechanism. Adaptation

to chronic NO_2 exposures was also found. Currently the inhalation facilities are being used for studies of the effects of diesel fuel aerosols on pulmonary function and systemic effects.

Studies on the ultrastructural changes in carcinogen treated respiratory tract cells have shown that certain PAHs cause persistent changes in the cytoskeletal-adhesive complex.

Some separate immunological studies complement the search for markers of pulmonary neoplasia. A tumor surface protein that has not been found in normal lungs or other tumor types has been identified in lung tumors of mice. Some evidence suggests that the protein results from activity of cellular genes which are not expressed in normal cells. Such proteins that are expressed by tumor cells provide a potential method for producing specific antibodies that could be directed to tumor target cells. Monocloned antibodies to lung tumor proteins are being produced with hybridomas to test the potential of such a therapeutic approach.

Other studies of the immune system are concerned with the determination of its role in chemical carcinogenesis and the immunosuppressive effects of environmental agents.

Unfortunately the hematology programs that were once such a vital feature of the Division's programs are now restricted to megakaryocytopoiesis and its regulation. It has been shown that the plasma levels of a humoral factor, presumably analogous to erythropoietin, rises in platelet-deficient rats.

The toxicology program is still evolving but has developed studies of the mechanisms of acute and chronic lung damage and of cardiovascular and other effects of metals as two major activities. The sequelae of damage to specific cell populations of the respiratory tract has given an insight into the possible mechanism of chronic lung disease. Furthermore, these studies have revealed how sequential exposure to differential agents may result in lesions that are distinct from those that would occur with exposure to a single agent. The study of the effects of environmental agents on cardiovascular disease includes analysis of the coal-associated trace element, cadmium, and its capacity to induce hypertension. Atherosclerosis and hypertension have been induced in pigeons after exposure to drinking water containing cadmium (0.6 ppm) or lead (0.8 ppm). In an attempt to resolve the mechanisms of cadmium-induced hypertension, it has been observed that cadmium causes an increase in plasma norepinephrine of both rats and pigeons.

While studies in radiation carcinogenesis have resulted in an increase in information about time-dose relationships and neutron carcinogenesis, they clearly indicate the need for further experiments. For example, it is now clear that the assumption of linearity of the dose-response relationships, at least over a considerable dose range of neutrons, is not valid. It is not known precisely how the various physical and biological factors influence the dose-response curve, but it is clear that the curve bends over at doses as low as 10-15 rad. The development of an *in vitro-in vivo* mammary cell system will facilitate investigations of the competing factors, namely, transformation and cell killing. The extensive dose-rate experiments done here over many years have provided most of the data on experimental radiation carcinogenesis used in a recent National Council for Radiation Protection report that exposure to low LET-radiation at low dose rates was significantly less tumorigenic than at high dose rates.

The radiation studies will also concentrate on problems important to the estimate of human risk. First are the effects of interaction of chemicals and radiation and the influence of dose rate on carcinogenic effects of chemicals. A second area of interest is the

examination of methods of extrapolation of animal data to human risk. The limits of a simple generalization are being tested, the generalization being that the ratio of incidence induced per rad of radiation to the natural incidence of a specific type of cancer is similar across species.

Radiation Biology

RADIATION CARCINOGENESIS

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The objective of this research program is to examine dose response and time-dose relationships in carcinogenesis and to examine the mechanistic basis for these relationships. The primary emphasis of the research within this program is on radiation carcinogenesis, although studies on dose-response and time-dose relationships with chemical agents have recently been initiated. In these studies two murine model systems have been used: (i) lung adenocarcinomas, (ii) mammary adenocarcinomas. Both of these tumor types are readily induced in BALB/c mice after treatment with carcinogenic agents. Recently the greatest amount of effort has been directed toward studies utilizing the murine mammary tumor system. The current research within this program may be conveniently divided into three areas: (i) neutron carcinogenesis; (ii) dose and dose-rate effects on the carcinogenic potential of chemicals, and (iii) carcinogenic interactions of radiation and chemicals.

Neutron Carcinogenesis. The largest research effort is directed toward the study of carcinogenesis after exposure to neutrons. Recent research findings on the carcinogenic effects of neutrons have made it apparent that dose response relationships and RBE dose relationships are not adequately defined. In addition, recent data have suggested that protraction may enhance the mutagenic and carcinogenic effects of neutrons. The objective of the project is to define the dose response relationships, the RBE-dose relationship, and the dose-rate relationship for the induction of alveolar cell lung adenocarcinomas and mammary adenocarcinomas and to understand their mechanistic basis. For the mechanistic studies we are investigating growth and differentiation characteristics of mammary cells in gland free fat pads. This approach allows the quantitation of both transformation and cell killing as well as the study of repair, the persistence of transformation and the role of cell-cell interaction on expression of transformation.

Dose and Dose Rate Effects on the Carcinogenic Potential of Chemicals. Because of the many factors involved in tumorigenesis, interpretation of differences in the effects of different carcinogens or rates of exposure on tumorigenesis may be confounded by their effects on factors influencing tumor expression rather than the induction of initial events. Therefore, tumor formation may not accurately reflect the full carcinogenic potential of the treatment. For an understanding of risks and extrapolation across species, it is essential to be able to study the initial events separately from factors involved in expression. This information on initial events more accurately reflects the carcinogenic potential of the treatment.

This study is designed to develop and utilize a relatively rapid quantitative method for assessing the carcinogenic potential of mammary epithelial cells. The principles for this method are well established but have not previously been applied to the problem of carcinogenic risk evaluation. By injecting dissociated mammary cells into gland-free fat

pads. We can quantitate both cytotoxicity and transformation. From experiments using this approach, information on dose response and time-dose relationships for 7-12-dimethylbenz[a]anthracene is being obtained, and comparative information on the carcinogenic potential of different compounds is being derived. Such information for initial events is essential for an understanding of risks and extrapolation of data across species.

Carcinogenic Interactions of Radiation and Chemicals. The potential risks that may result from interactions of low-dose and dose-rate exposures of ionizing radiation with chemical carcinogens cannot be assessed from presently available data. Although chemicals and radiation are additive in their carcinogenic effects at high doses, it is not known whether this is true at low doses and dose rates. In fact, some data suggest that an assumption of additivity is incorrect and may underestimate the potential risks. The objective of this project is to examine the nature of the interactions between radiation and chemicals at low doses and dose rates with particular emphasis on the potential tumor enhancing or cocarcinogenic activity of low-dose-rate radiation exposures. Specifically this research examines (i) whether the carcinogenic effectiveness of the radiation dose influences the nature of the interactions between radiation and a chemical carcinogen, (ii) the nature of the interaction of a chemical carcinogen and low-dose-rate radiation, and (iii) the influence of radiation quality on these interactions. To approach these questions, in vivo mammary tumorigenesis and studies with mammary cells and tissues in gland-free fat pads are used.

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ULTRAVIOLET RADIATION CARCINOGENESIS

R. J. H. Fry R. D. Ley

Skin cancer is by far the commonest cancer in the white population of the United States and ultraviolet radiation (UVR) is the most important etiological factor.

The current studies stem from experiments that investigated the role of photoproducts after exposure to psoralen and UVR of various spectra. As a result of these experiments, we have developed methods that we believe make it possible to carry out quantitative studies on UVR carcinogenesis and allow determination separately of the role of factors that influence initiation and expression. The studies fall into three separate but related areas.

The Development of Radioimmunoassay Techniques for the Assay of UVR-induced DNA Damage Precise determinations of the amount of damage induced in target cells of the epidermis are essential for meaningful dose-response studies of skin carcinogenesis. Dosimetry is difficult since most skin carcinogenesis experiments require multiple exposures to a chemical or physical carcinogen. It cannot be assumed that equivalent levels of damage will be induced by each exposure. Initial exposures may alter epidermal thickness, which in turn will result in decreased penetration of the carcinogen during subsequent exposures. Therefore, it is important to determine (i) whether an increase in level of carcinogen applied at the surface of the skin results in increased damage to target cells in the epidermis, and (ii) whether the dose response for damage induction is maintained during the course of the tumor-induction experiment. Without this information, any dose response observed for the induction of tumors may be difficult, if not impossible, to interpret. Interspecies comparisons of tumor-induction response based on good dosimetry may eventually allow risk estimates for skin carcinogenesis to be made for man.

Meaningful determinations of the above dose parameters will require the ability to measure the induction, distribution, and fate of DNA damage in epithelial cells exposed *in vivo* at levels similar to those induced during cancer induction studies. Radioimmunoassays (RIA) may provide the necessary sensitivity and specificity to make these determinations feasible. Our studies to date indicate that RIA for DNA damage induced in epithelial cells have levels of detection that will surpass the sensitivities of currently used techniques. RIA have been developed that not only measure one type of DNA lesion, i.e., pyrimidine dimers, but can also distinguish between thymine-thymine dimers and cytosine-containing dimers. This is evidence for a high degree of specificity of RIA for DNA damage.

The Determination of (i) Dose and Time-dose Relationships for UVR-induced Skin Carcinogenesis, (ii) Age and Strain and Species Dependency for Susceptibility. In this segment of the program the role of specific DNA damage and its repair and the role of the immune system (in collaboration with E. H. Perkins) are investigated (R. J. H. Fry and R. D. Ley). In studies of the UVR induction of skin cancer, it is necessary to use multiple exposures. We have shown that a relatively small number of fractions of UVR (280-400 nm) can be used if 12-*O*-tetradecanoylphorbol-13-acetate is administered repeatedly after the last exposure. Using doses/fraction below the level that cause hyperplasia, we have determined the dose response for the induction of initiation events and a linear dose response relationship cannot be excluded.

The Development of New Mouse Model Systems for the Study of UVR Carcinogenesis and the Investigation of Some of the Factors that Influence Tumorigenesis in the Skin. This part of

the program stems from that invaluable resource — the Russell's mouse facility. The studies are a collaborative effort involving L. B. Russell, J. M. Holland, E. H. Perkins, R. D. Ley, and R. J. M. Fry.

Four autosomal dominant mutants producing a hairless phenotype have been identified: poor fur, fur loss, near naked, and one for which no name has yet been assigned. The four mutations are being backcrossed to two inbred strains (BALB/c and C57BL/6). Since we have colonies of athymic nude mice co-isogenic with BALB/c and C57BL/6, eventually it will be possible to determine the comparative susceptibility for UVR cancer induction in haired, hairless, and nude BALB/c and C57BL/6 mice. Thus, the importance of pyrimidine dimer induction and repair, follicular cells, melanin and immune competence in UVR-induced skin carcinogenesis can be investigated.

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Chemical Carcinogenesis

MECHANISM OF ACTION OF CHEMICAL CARCINOGENS

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Chemicals that cause cancer form a diverse class of compounds of widely different structures that include polyaromatic, heterocyclic, and aliphatic compounds. While these substances may have major differences in their chemical and physical properties, it is believed that they are inert as found in the environment and require some degree of metabolic activation by susceptible cells to exert their carcinogenic properties. To date the only general statement which can be made regarding this diverse group of substances is their ability to form an electrophilic reagent via metabolism which reacts readily with cellular nucleophiles. It can be assumed that this process is the normal detoxification mechanism that is designed to inactivate xenobiotics. In the case of chemical carcinogens, the process has inadvertently formed a highly reactive electrophilic reagent that alkylates susceptible macromolecules before they can be hydrated and removed as a water-soluble conjugate.

Our work has centered around several facets of the activation and detoxification process with major emphasis on the polyaromatic class of hydrocarbons. At the present time, it is assumed that the transforming event in a susceptible cell is modulated by the binding of a reactive diol-epoxide intermediate of benzo[a]pyrene to DNA with guanine as the major binding moiety. However, there is no viable mechanism to involve this stereo-specific binding to the malignant event since identical binding occurs in all cells studied, both susceptible and resistant to malignant transformation by benzo[a]pyrene.

Our research is focused upon understanding the processing of the carcinogen from the moment it is presented to the cell and is acted upon by the microsomal and cytoplasmic enzyme systems until it is either excreted as a detoxification product or ultimately bound to one or

all of the components of chromatin. We are studying the partition of the activated intermediates in the cytoplasm and the surrounding medium as well as those intermediates which may be found in the nuclear sap. In addition to using susceptible and resistant cells for this process, we have utilized various chemical isomers where possible. We have observed a number of biochemical differences between carcinogenic benzo[a]pyrene and its noncarcinogenic positional isomer benzo[e]pyrene, and we have approached this problem by isolating and identifying organic-soluble and water-soluble intermediates, both intracellular and extracellular, and investigating the extent of the binding to nuclear macromolecules. We have been able to show the probable cause of the inactivity of benzo[e]pyrene as a carcinogen, both in intact cellular systems as well as with the purified P-450 monooxygenase enzyme complex.

Another chemical probe we have utilized is 2-OH-B[a]P which is the only carcinogenic phenol of the twelve B[a]P phenols. Our studies have shown that 2-OH-B[a]P is not readily metabolized in cell-free systems and requires intact cells for activation, and that it possesses a far longer residence time within the cell than any of the other known phenols. We have isolated and partially identified the major metabolic product of 2-OH-B[a]P in hamster embryo fibroblasts and have found another major metabolite in human and rodent skin cells not present in rodent fibroblast systems to any great extent. We are currently attempting to isolate enough of this material for careful chemical characterization. Our most recent progress has shown evidence for an additional level of molecular discrimination within the nucleus by observing a high degree of specificity for the binding of new active intermediates of benzo[a]pyrene to nuclear proteins. We have observed the possible appearance of an additional diol-epoxide (9,10-diol-7,8-oxide) not readily found as a cytoplasmic product which is possibly formed in the nucleus which binds to nonhistones as opposed to the opposite diol-epoxide (7,8-diol-9,10-oxide) which is known to bind predominantly to guanine in DNA as well as two of the core histones. These results have shown a continual level of discrimination in cellular processing of potential carcinogenic intermediates. We are currently developing high-pressure liquid chromatographic techniques for separation and identification of the probable tetrol products of this diol-epoxide. Our program is beginning to study the possible role of azaarenes as environmental carcinogens since these compounds are major products of diesel fuel combustion. We hope to determine if these molecules, which contain one heteroatom, follow the same microsomal activation and detoxification patterns that the polyaromatic compounds undergo. We will attempt to study total cellular processing of aza- and nitroaromatic compounds that appear as potential carcinogens and mutagens.

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EFFECTS OF CHEMICAL CARCINOGENS ON NUCLEIC ACIDS

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The biochemical steps leading to malignant transformation of cells are not well understood. The overall process, however, may be divided into three stages. the damage to the cell by some agent, the partial recovery of the cell, and the progression of that transformed cell to malignancy. This last process requires the transformed cell to increase in number. It is the propensity to undergo unregulated growth that characterizes the cancer cell. There are probably many ways for cells to bypass the normal growth regulation. We have begun studies of the altered behavior of nucleic acid metabolism as a potential site for loss of growth regulation since continued production of RNA and DNA are required for continued cell division.

We have developed procedures that permit nondestructive measurements (1-3) of cellular changes in nucleic acid metabolism in rodent and human cell cultures after exposure to benzo[a]pyrene. We have discovered a physiologically regulated excretion of Urd and Cyd by cells in culture and have established the biochemical events that govern the phenomenon. The key event is the metabolic isolation of the cytoplasmic nucleoside polyphosphate pool that is created by a very low or absent UMP-ATP transphosphorylase activity. This phenomenon also provides a biochemical basis for the known compartmentation of nucleotide precursors for nucleic acid synthesis.

With this new understanding of how the pyrimidine nucleoside pools flow into RNA, DNA, or UXP (CXP), we have measured specific activity changes in extracellular Urd, Cyd and Yrd and

find we can determine the rates of de novo synthesis of UMP, CMP, RNA turnover and the availability of the cytoplasmic nucleoside phosphates (UXP, CXP) for RNA and DNA synthesis without destroying the cell.

We have also developed procedures for monitoring the biosynthesis of the several RNA and DNA populations using gel filtration and electrophoretic techniques. Application of these techniques has given us data that describe the specific effects of B[a]P on RNA and DNA metabolism, the rate of B[a]P adduct formation on each RNA population, and some encouragement that continuing this course will yield mechanistic information on altered nucleic acid metabolism accompanying malignant transformation.

As a result of our studies, we have discovered a complex rate of binding of B[a]P metabolites to RNA and DNA. The results are consistent, with the nucleus being the primary site of carcinogen-RNA interaction. In addition, this binding results in a perturbation of RNA processing and turnover. One end result of these reactions is an enhanced excretion of nucleosides that may be useful in the design of a short-term carcinogen assay.

We are continuing studies to establish the cellular basis for increased excretion of modified nucleosides by tumor-bearing humans and animals.

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IN VITRO CHEMICAL CARCINOGENESIS

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Our aim is to develop rodent and human cell culture systems for the study and identification of chemicals which initiate and/or promote tumor formation. We approach this by further developing our cell-mediated mutagenesis assays. In these assays, mutable Chinese hamster V79 or our newly developed, highly mutable human P3 cells are co-cultivated with cells, derived from various organs from experimental animals and humans, capable of metabolizing chemical carcinogens. During the co-cultivation, the reactive metabolites produced by the metabolizing cells mutate the V79 and P3 cells at different genetic loci such as HGPRT, Na^+/K^+ ATPase (ouabain resistance) and other newly developed markers such as mycophenolic acid and aphidocolin resistance. These sensitive assays have the potential of predicting degree of carcinogenicity as determined by a series of 15 nitrosamines with different degrees of carcinogenicity and are indicative of organ specificity as shown in the case of aflatoxin B_1, benzo[a]pyrene, and dimethylnitrosamine. In addition, introducing newly isolated error-prone mutants from the V79 cells will allow us to further increase both our understanding of the mechanism of mutagenesis and the sensitivity of our cell-mediated assay. We will continue our effort to identify the ultimate mutagenic metabolite of potent carcinogens as we did in the past in the case of benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene, and to search for markers of epithelial cell transformation in addition to γ-glutamyl-transpeptidase, which we found in a series of rat liver cell lines to be associated with

malignant cell transformation. Recently, our studies indicated that tumor promoting phorbol diesters can induce terminal differentiation in human promyelocytic leukemia and melanoma cells. Differentiation in the leukemia cells was determined by inhibition of cell growth, cell attachment, morphological maturation, phagocytosis, lysozyme and acid phosphatase activity, and, in the case of the melanoma cells, by inhibition of cell growth, stimulation of melanin synthesis, and dendrite-like structure formation. Differentiation in both cell systems could be detected at doses as low as 10^{-10}M of the potent tumor promoter, phorbol-12-myristate-13-acetate, and the degree of induction by a series of phorbol esters was correlated with their activity in the mouse skin. These cell systems may be useful for studying the control of cell growth and malignancy and perhaps serve as short-term assays for identifying potential tumor promoters. We are at the present time studying various biochemical changes, such as lipids and polyamine biosynthesis, that may be associated with the induction of differentiation in these cells.

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SKIN CARCINOGENESIS AND TUMOR PROMOTION

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The overall goal of this research program is to determine the mechanism(s) by which polycyclic aromatic hydrocarbon (PAH) carcinogens cause skin cancer. This is being pursued *in vivo* as well as *in vitro* using epidermal cells in culture. Skin carcinogenesis can be divided into at least two distinct stages, initiation and promotion, allowing each stage to be defined and studied individually. We have recently found that the tumor promotion stage can be further divided into two and possibly three stages, which should allow us to even better determine the important events and their sequential nature in promotion. Inhibitors and stimulators of tumor initiation are being employed to better define the critical event(s) in tumor initiation by PAH especially related to the critical nuclear interaction(s) by PAH. Modifiers of the tumor promotion stage are also being used to dissect out the critical events in promotion. We are specifically determining the importance of the induction of primitive stem cells (dark keratinocytes) as well as the role of prostaglandins (PG) and polyamines (PA) in promotion.

In Vitro Transformation of Epidermal Cells. *In vitro* transformation of epidermal cells from tumor sensitive mice (SENCAR) is performed using our new improved culture conditions which allow the epidermal cells to maintain their epithelial morphology even after being subcultured several times. These cells have extensive desmosomes and produce keratin as evidenced by staining with Rhodanile blue and the presence of two major proteins that migrate with keratin 1 and 2 from intact skin in NaDodSO$_4$ gel electrophoresis. In addition, as observed by electron microscopy, the subculturable epidermal cells produce a stratification *in vitro* very similar to that of *in vivo* epidermis. These epidermal cells are grown on a 3T3 feeder layer at 31°C in enriched Waymouth medium which is supplemented with 10% fetal calf serum with the addition of insulin (10 µg/ml), hydrocortisone (0.1 µg/ml), and epidermal growth factor (10 ng/ml). Using the above conditions, we have in a preliminary experiment transformed a primary culture and one of our nontumorigenic cell lines which gave rise to keratinizing squamous cell carcinomas when injected into nude mice. Our proposed goal is to develop not only a reliable and quantitative *in vitro* transformation system using epidermal cells from skin tumor sensitive mice, but also to develop a relevant one in which two-stage transformation is operational using phorbol-ester tumor promoters and one in which transformed cells give rise to keratinizing squamous cell carcinomas when injected into a syngeneic host.

Polycyclic Hydrocarbon Metabolism and Binding in Skin. The mechanism of carcinogenic action of PAH is being investigated through the use of metabolism, binding, and tumorigenicity studies. The results from our metabolism studies of benzo[*a*]pyrene (BP) and 7,12-dimethylbenz[*a*]anthracene (DMBA) in tumor sensitive and resistant mice as analyzed by high pressure liquid chromatography, and our tumorigenesis studies of various BP, dibenz[*a*,*h*]anthracene, DMBA, chrysene, 7-methylbenz[*a*]anthracene and benzo[*e*]pyrene metabo-

lites and derivatives suggest that diol-epoxides in the "bay region" of these hydrocarbons are important in their tumorigenic activity. Our binding studies suggest that BP-diol-epoxide interacts to a greater extent and with some degree of specificity to the internucleosomal region of chromatin. Approximately 85% of the binding of BP-diol-epoxide to chromatin is to DNA, with a high specific activity to G-C rich regions of DNA. Through the use of inhibitors of BP and DMBA tumorigenicity, our data suggest that the interactions of BP-diol-epoxide to the exocyclic nitrogen of guanines and adenines are important adducts in the initiation of the carcinogenic process. Further studies are under way to determine the specificity of binding BP-diol-epoxide to guanines and adenines, as well as specific sequences in DNA. In addition, the importance of BP-diol-epoxide interaction with histones and nonhistone proteins is being investigated.

Multistage Promotion. Various modifiers of the tumor promotion process have been very useful in our understanding of the mechanism(s) of tumor promotion. The anti-inflammatory steroids, retinoids, protease inhibitors, and PG and PA biosynthesis inhibitors are potent inhibitors of tumor promotion. The phorbol-ester tumor promoters have been shown to have several cellular and biochemical effects on the skin. Of all the observed phorbol ester related effects on the skin, the induction of epidermal cell proliferation, PA, PG, and dark basal keratinocytes, as well as other embryonic conditions, appear to correlate the best. Mezerein, a weak promoter, was found to induce many cellular and biochemical changes similar to 12-O-tetradecanoylphorbol-13-acetate (TPA), especially epidermal hyperplasia and PA; however, it was not a potent inducer of dark cells. We recently found that promotion could be divided into two stages. The first stage (I) is brought about by limited treatment of TPA and the second stage (II) by repetitive applications of mezerein. The anti-inflammatory steroid fluocinolone acetonide (FA) was found to be a potent inhibitor of stages I and II. Retinoic acid (RA) was ineffective in stage I but was a potent inhibitor of stage II promotion, whereas tosyl phenylalanine chloromethyl ketone (TPCK) specifically inhibited stage I. In addition, FA effectively counteracted the TPA-induced cell proliferation and dark basal keratinocytes but had very little effect on PA, whereas RA had no effect on TPA-induced cell proliferation and dark cells but is a potent inhibitor of TPA-induced ornithine decarboxylase (ODC) activity and subsequent putrescine formation. TPCK had no effect on the TPA induced cell proliferation and PA but counteracted the appearance of the dark cells. These results provide additional evidence for the importance of dark basal keratinocytes (primitive stem cells) in stage I of promotion and indicate that most of the other cellular and biochemical responses normally associated with promotion (such as PA) are actually associated with stage II of promotion.

Role of Polyamines in Tumor Promotion. Promoter specific induction of ODC and ensuing PA biosynthesis was examined. We found that elevated ODC activity and PA levels could be dissociated from tumor-promoter-induced proliferative events. By the use of inhibitors and other irritant-like compounds we determined, however, that PA are associated with a second stage or aspect of tumor promotion. Further, putrescine per se is nonpromoting but enhanced TPA-induced papilloma formation. Highly specific inhibitors of PA biosynthesis do, in fact, lower papilloma incidence. Also, besides finding that the concentration of putrescine was extremely high in papillomas generated in a two-stage protocol, we determined that putrescine was strongly bound or cross-linked with epidermal macromolecules. This may be via the transglutaminase(s) or oxidation. In vivo and in vitro C^{14} spermine was degraded to both putrescine and spermidine. The effect of TPA on these metabolism changes is under study.

Lastly, we determined that there is a strong association of TPA-induced (i) alteration in differentiation with (ii) changes in PA biosynthesis.

Role of Prostaglandins in Tumor Promotion. The mechanisms of action of chemical carcinogenesis in the skin are being studied using the SENCAR mouse. This process can be divided into two distinct stages known as initiation and promotion.

Since it is known that an inflammatory state is associated with the tumor-promotion stage of carcinogenesis, we have studied the effects of some of the mediators of inflammation. In particular, some of the PG have been shown to have either an inhibitory of enhancing effect on tumor promotion. Some studies have been carried out using specific inhibitors of the PG biosynthetic pathway which have further suggested the intimate involvement of the PGs in promotion.

Further work is needed to determine how and in what manner tumor promoters disturb the normal balance of products from the PG biosynthetic pathway and how these agents alter tumor production. In-depth studies are being pursued on the role of PG primarily through the use of inhibitors specific for the various branches of the PG biosynthetic pathway, including the thromboxanes, the hydroperoxy fatty acids, and the PG proper. The effect of these metabolites on tumor promotion are also being investigated. Preliminary results have revealed that PG synthesis inhibitors phenidone and 5,8,11-14-eicosatetrayonic acid are potent inhibitors of tumor promotion. In addition, two thromboxane synthetase inhibitors (RO 22-3581 and RO-22-3582) were found to be potent inhibitors of tumor promotion.

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Respiratory Carcinogenesis

NEOPLASTIC TRANSFORMATION IN TRACHEAL EPITHELIUM

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Markers of Neoplastic Development in Respiratory Epithelium. In this program, several in vivo-in vitro and in vitro model systems have been developed and are utilized to define and quantify cellular changes that identify particular stages in the evolution of neoplasia in respiratory epithelium. In an in vivo-in vitro system, tracheal implants are exposed to known doses of carcinogen for pre-selected periods of time. Primary epithelial cell cultures are then established from explants cut from the implants. Early changes in carcinogen-exposed cells are identified by an increased in vitro growth capacity, i.e., survival under suboptimal culture conditions followed by subculturability of the primary cultures. Dose-dependent effects were detected as an increase in number of surviving cultures, decrease in time during subculture to anchorage-independent growth (growth in agar), as well as to tumorigenicity. Experiments in progress attempt to correlate genetic and other cellular and biochemical markers in cell populations derived from specific lesions identified non-destructively by exfoliative cytology in organ culture. In this way, highly selected pre-neoplastic cell populations can be studied.

In the in vitro systems, either tracheal organ cultures, primary cell cultures, or established tracheal epithelial cell lines are exposed to carcinogen. With the first in vitro system, we have demonstrated that a "preneoplastic" marker in primary cell cultures established from the organ cultures is morphologically altered foci of small, packed, highly proliferating cells. This alteration pre-dates subculturability and possible acquisition of tumorigenicity. The tumor promoter tetradecanoyl-phorbol-acetate induces similar foci, but not tumorigenicity. In combination with the carcinogen MNNG, the promoter enhances foci formation and tumorigenicity. Further experiments with the tracheal organ culture and the primary cell culture are in progress to determine carcinogen-dose effects on the progression to neoplasia. We have also demonstrated in vitro transformation of a nontumorigenic tracheal epithelial cell line. Carcinogen metabolism studies are carried out in concert with transformation in these cells. Experiments are in progress to develop a highly quantitative in vitro transformation system with clones of these cells based on alteration in growth requirements.

Modulation of Growth Differentiation and Transformation in Tracheal Epithelium by Vitamin A. The main objective of this program is to study the control of growth and differentiation in normal and carcinogen-altered tracheal epithelial cells. To have a biochemical marker of differentiation, procedures were first developed to isolate and purify high-molecular-weight mucous glycoproteins, one of the specialized products of the normal respiratory epithelium. The source of the material was the luminal contents of tracheal implants which accumulate large amounts of secretions. These procedures were then applied in comparative morphometric and biochemical studies of the effects of vitamin A on the synthesis and secretion of these glycoproteins by long-term tracheal organ cultures. These cultures exhibit a normal mucociliary epithelium or go through a metaplastic change to a keratinizing

squamous epithelium based solely on the presence or absence of vitamin A in serum-free medium. The study of the effects of vitamin A has also been taken to the cell culture level. One approach was to determine the response to vitamin A *in vitro* of two epithelial cell lines, one cloned from a mucus secreting adenocarcinoma and the other from a keratinizing squamous cell carcinoma. Both cell lines synthesize little mucous glycoprotein *in vitro* in the absence of vitamin A. Supplementing the medium with vitamin A inhibited growth only slightly, but stimulated the synthesis and secretion of mucous glycoproteins in both cell lines. However, 20X more glycoproteins were synthesized by the cell line from the adenocarcinoma indicating that these cells have a fixed greater capacity to respond to the vitamin. We have also determined that vitamin A acts as a growth factor in some epithelial cell lines by stimulating cell proliferation, increasing cell saturation density and turnover of the cell population. These multifaceted effects of the vitamin will be followed in epithelial cell lines during carcinogen exposure and neoplastic transformation *in vitro* and should offer the opportunity to delve further into the molecular basis of the control of growth, differentiation, and transformation in these epithelial cell systems.

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NORMAL AND NEOPLASTIC GROWTH IN EPITHELIAL CELLS

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In Vitro Studies on the Dynamics of Neoplastic Development In Vivo. The development of neoplastic disease *in vivo* is characterized by a long asymptomatic latency period. The length of this latency period is dependent on the dose, dose rate, and potency of carcinogenic insult. We have developed an *in vitro* technique referred to as the epithelial focus (EF)-assay whereby quantitative and qualitative changes taking place soon after carcinogen exposure can be monitored. Cells giving rise *in vitro* to EF which survive and proliferate

when seeded into culture dishes were frequently observed immediately after carcinogen-exposure in rat trachea, esophagus, and lung. They were rarely observed in cultures established from nonexposed control tissues. Three types of EF could be distinguished experimentally: EF which could not be subcultured (EF_0); EF that could be subcultured (EF_S); and EF_S which did not grow in agarose ($EF_{S,ag-}$); and EF_S which did grow in soft agarose ($EF_{S,ag+}$). We have been investigating the effect of carcinogen(DMBA) dose and time post-exposure on the dynamics of neoplastic development in implanted rat tracheal epithelium as determined by changes occurring in the EF, EF_0, EF_S, $EF_{S,ag+}$, and $EF_{S,ag-}$ cell pools. Over a wide range of DMBA doses (5-350 μg) at times 0-4 months post-exposure, essentially all (80-100%) of tracheas contained $1\text{-}10^3$ EF-forming cell units. Over this dose range no systematic dose or time dependency was observed for EF frequency. However the frequency of $EF_{S,ag+}$ was clearly dose dependent. No $EF_{S,ag+}$ occurred at or below doses of 50 μg DMBA. At 165 μg and 350 μg, the frequency of $EF_{S,ag+}$ was 10% at 0 time. No significant increase in $EF_{S,ag+}$ with time was detected at 165 μg DMBA. At 350 μg a significant increase in the proportion of $EF_{S,ag+}$ was observed between 0-4 months (65% $EF_{S,ag+}$). Similar studies have been initiated on the effect of promoters and other carcinogenic agents (e.g., radiation) on the dynamics of neoplastic development in "initiated" cell populations.

In Vivo Studies of the Growth and Differentiation of Normal and Cultured Preneoplastic Tracheal Epithelial Cells. The development of neoplasia is presumed to occur as a multistage process in which "initiated" cells gradually acquire neoplastic characteristics. For a number of reasons it has been difficult to obtain conclusive evidence for this developmental hypothesis since it is generally impossible to take repeated samples from a "lesion" presumed to be composed of "preneoplastic" cells *in vivo*. In cell culture, one can obtain repeated samples of the same cell population over extended periods of time; however, this approach has other limitations. We have developed a technique referred to as tracheal repopulation whereby the growth of normal and preneoplastic epithelial cells previously isolated *in vitro* can be studied *in vivo* in de-epithelialized tracheal implants. This model attempts to incorporate the desirable features of both systems for studying neoplastic progression. Tracheas denuded of their epithelium are inoculated with suspensions of normal or preneoplastic rat tracheal epithelium and then implanted subdermally into isogenic recipients. Within 2 weeks of implantation, a new epithelial lining is established. The histologic appearance of the epithelium varied: normal cells yielded a mucociliary epithelium; preneoplastic cells yielded a mucociliary, undifferentiated or squamous epithelium; neoplastic cells yielded an invasive undifferentiated, squamous or adenomatous epithelium. The substrata on which altered cell populations are grown *in vivo* does not affect their growth or differentiation. Normal cells, although they survive and differentiate on nonviable freeze-thawed stroma, do not survive on stromal tissues which have been heated, fixed in ethanol, or predigested with collagenase. These "*in vivo* culture" techniques will be used to further investigate sequential changes in growth and differentiation of isolated carcinogen-exposed epithelial cell populations. The applicability of these techniques to other tissues and other species will also be investigated.

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RESPIRATORY NEOPLASIA

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Morphogenesis of Chemically Induced Respiratory Neoplasia. Tracheal implants exposed to dimethylbenz[a]anthracene (150-200 µg) for 4 weeks develop numerous metaplastic and dysplastic epithelial lesions. The chronological appearance and relationship of these lesions with invasive carcinoma were studied in our laboratory. It was found that 4 months after cessation of carcinogen exposure, 72% of the tracheas contained lesions. This percentage decreased markedly, and over the subsequent 8 months only 44% of the tracheas exhibited lesions. Notwithstanding this total decline, the percentage of tracheas with advanced lesions (atypical dysplasias) did not decrease. The advanced lesions persisted for long periods of time, but only a relatively small percentage evolved into invasive carcinoma (approximately 1 out of 4), and some also appeared to regress. Most lesions without atypia seem to ultimately regress.

A search for morphological markers of the putative preneoplastic lesions showed an increased relative amount of acid mucosubstances in the lesions with severe atypia. In addition, progressive changes in ^{3}H-thymidine labeling index and nuclear cytoplasmic ratios were detected. At the ultrastructural level, the appearance of dark epithelial cells was noted. These cells increased in number with increasing degrees of atypia, and well defined ultrastructural differences could be seen between the carcinogen-induced dark cells and the dark cells seen in squamous metaplasias produced by formaldehyde irritation or vitamin A-deficiency.

Studies are presently in progress in order to determine the nature of the epithelial dark cells and to what extent they participate in the process of neoplastic progression.

Mechanisms of Chemical Carcinogenesis in Heterotopic Tracheal Implants. The rat tracheal implant (t.i.) model was developed some years ago in this laboratory by P. Nettesheim, J. Kendrick, and R. Griesemer. Since then, several chemical carcinogens have been used to expose *in vivo* the respiratory epithelium yielding carcinogen-altered epithelium, focal epithelial lesions of probable preneoplastic nature, and carcinomas. These experiments were performed by inserting in the lumen of the t.i. bees-wax pellets containing measured amounts of either dimethylbenzanthracene (DMBA) or benzo[a]pyrene (BP). The homogeneous exposure to the carcinogen, a precise determinationof the release dose, as well as the lack of interference with the normal respiratory physiology of the host animals are the main advantages of this model, which allowed our group to determine the dose-dependent tumorigenic effect of DMBA and BP on the respiratory tract epithelium.

Recently, D. Topping and P. Nettesheim have described the tumorigenic effect of chrysotile A asbestos as well as the tumor-enhancing effects of 12-O-tetradecanoyl-phorbol-13 acetate (TPA) and asbestos on the tracheal epithelium previously exposed to low doses of

DMBA. Several experiments are now under way in order to further elucidate the mechanisms of cocarcinogens and tumor promotion using the t.i. model. In this context, the following questions are addressed: (i) Can respiratory epithelial cells, initiated with subtumorigenic doses of carcinogen, be promoted with TPA? (ii) What is the influence of the initiator and promoter doses in this two-stage carcinogenesis model? (iii) What is the influence of dose rate of DMBA exposure on the two-stage carcinogenesis protocol? (iv) What is the effect of certain particulate and fibrillar material (such as asbestos, glass fibers, nickel subsulfide, and arsenic trioxide) when used as initiators or promoters?

The characterization of the circumstances and mechanisms under which initiated respiratory epithelial cells respond to secondary stimuli will also be studied in combined *in vivo-in vitro* experiments in which the progression of neoplastic development can be determined with high sensitivity prior to the appearance of the clinically or histopathologically detectable neoplasia.

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CONTROLLED RELEASE OF CARCINOGENS, COCARCINOGENS, AND PROMOTERS OF TUMOR INDUCTION IN HETEROTOPIC TRACHEAL TRANSPLANTS

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In the tracheal transplant model, the trachea is surgically removed from one rodent and transplanted into the scapular region under the skin of another isogenic host. The transplant reestablishes itself in the host in about four weeks and continues living like a normal trachea. This model offers obvious advantages over the conventional approaches to the investigation of respiratory tract carcinogenesis by intratracheal injection or instillation

methods in that here a defined part of the respiratory tract can be exposed to carcinogens, cocarcinogens, and promoters in a controlled and quantitative fashion. Consequently, the development of systems for controlled and predictable release of these agents assumes paramount importance for the success of the tracheal transplant model for studies in tumor induction, tumor promotion, cocarcinogenesis, and dose-response relationships.

Different approaches have been successfully used for controlled release of carcinogens in vivo. Single exposures can be given by incorporating these agents in gelatin pellets. Continuous exposure over a period of 4-24 weeks can be given by the use of monolithic cylindrical pellets using beeswax or cholesterol-beeswax (9:1). Polycyclic aromatic hydrocarbons and 12-O-tetradecanoylphorbol-13-acetate can be released in a controlled fashion in this manner. Arsenic can be released through silastic tubing. In all of these devices release of the active agents is controlled by diffusion through the matrix material. For controlled release of particulates such as Ni_3S_2, MnO_2, asbestos, etc., we are planning to use biodegradable matrix. It is expected that as the pellet degrades in vivo these particulates will be released exposing the tracheal epithelium in a uniform manner.

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ULTRASTRUCTURAL CHANGES IN LUNG EPITHELIUM

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It is relatively expensive and time consuming to carry out long-term studies at near-ambient levels of toxic and carcinogenic agents. However, the results of such studies cannot be duplicated by short-term exposures to equivalent total doses of the agents. The correlation between short-term and long-term responses can be expected to be particularly poor in cases of delayed or cumulative effects. We are interested in these effects in the respiratory tract, which is the immediate target of airborne agents.

We are comparing the short-term and long-term effects of the carcinogens 7,12-dimethylbenz[a]anthracene (DMBA) and benzo[a]pyrene (BP) on the upper respiratory tract epithelium. The morphological lesions produced by DMBA are partially reversed in general but become progressively more severe in focal areas. The ether-linked lipids, which are related to glycolytic metabolism and appear at high levels in carcinomas of the respiratory tract, are also elevated after DMBA treatment. Although the levels decline, they remain higher than in untreated tissues up to the time when dysplastic lesions appear. In DMBA initiated cell lines, which have an indeterminate life-span in vitro, the shapes of cells change in parallel with increases in anchorage-independence. Shape factors and nuclear-cytoplasmic ratios are also altered in DMBA-induced dysplastic lesions of the tracheal epithelium. Recently we discovered that cells derived from BP-induced tumors also have a perturbed keratin substructure when compared to less tumorigenic and nontumorigenic respiratory tract epithelial cells. Thus long-term effects of carcinogens in the respiratory tract are expressed at the cellular level as persistent alterations in glucose metabolism and in the cytoskeletal-adhesive complex.

We are also conducting ultrastructural studies of the alterations caused by chronic passive tobacco smoke exposure of rats. There are two alterations of particular importance that have not been described in the numerous previous reports on the morphological effects of smoke exposure. One is the presence of crystalloid structures in the epithelial cells lining the airways, including the presumptive target cells for carcinogens, and the second is that degradative changes occur in the basement membrane underlying the lung parenchymal epithelium. These changes provide alternative possibilities for physical, in addition to chemical, mechanisms of carcinogenesis and for interstitial injury, in addition to frank type I cell death, in fibrotic change respectively.

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Mammalian Toxicology

TOXICOLOGY

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Mechanisms of Acute and Chronic Lung Damage. The main thrust of the program in experimental toxicology is to study and to define pathogenetic principles underlying the development of lung lesions. Acute death of cells lining the alveoli and the small airways may be caused both by inhalation agents (e.g., oxygen, ozone, nitrogen oxide, 3-methylfuran) and by bloodborne agents (e.g., butylated hydroxytoluene, cyclophosphamide, methylcyclopentadienyl manganese tricarbonyl, bleomycin). Exposure to any of these agents will usually cause an initial destruction of epithelial cells followed by a proliferative response resulting in eventual tissue recovery. We have found that if tissue recovery is interfered with by a second toxic agent diffuse fibrosis of the lung develops and persists up to one year. Acute interactions between two toxic agents in the lung may thus result in a biological response which is both quantitatively and qualitatively different from the effect produced by individual agents alone. A damaged lung is also more susceptible to toxic inhalants than is a normal one. Our observations provide a possible explanation for the development of such

human diseases as adult respiratory distress syndrome and diffuse interstitial fibrosis. The experimental models developed allow us to examine whether such lesions can be prevented or ameliorated by using anti-inflammatory and anti-fibrotic therapeutic agents.

Carcinogenesis of a Common Air Pollutant. 3-Methylfuran is a major atmospheric contaminant produced by photooxidation and degradation of volatile hydrocarbons such as isoprene and terpene; it has been identified during a smog alert in Washington, D.C. In collaboration with Dr. Michael R. Boyd, National Cancer Institute, we have initiated a series of long-term inhalation studies in mice, hamsters, and rats. Exposure to 3-methylfuran causes selective damage to the Clara cells in the small airways. It is a potent alkylating agent and the present experiments are designed to establish whether 3-methylfuran has carcinogenic potential.

Validation of Mouse Lung Adenoma Assay. Several mouse strains, particularly A/J and Swiss-Webster mice, develop multiple lung tumors following single or repeated intraperitoneal injections of carcinogens. The mouse lung adenoma test has been suggested as a possible short-term *in vivo* bioassay for carcinogenesis. We are presently evaluating a number of unknown chemicals in this system; the same compounds are being evaluated in another laboratory with a similar procedure. The data from the two laboratories will be compared to determine where there is good agreement between laboratories. At the same time, we are collecting information on spontaneous tumor incidence in A/J mice, histopathology of tumors, dose response and time response to known carcinogens such as urethan, benzo[a]pyrene and 4-nitroquinolineoxide. We are also examining whether it is possible to enhance the sensitivity of the test by using an agent (butylated hydroxytoluene) that is known to promote adenoma formation in mouse lung.

Toxicology of Selected Crude and Refined Shale Oil Substances. Bulk samples of Paraho shale oil and its derivatives, received from ORNL sample repository, are being tested in selected acute toxicity assays. The procedures followed are the ones adopted by many toxicology laboratories involved in safety evaluation. The following tests are routinely being used: acute oral and intraperitoneal toxicity in mice and determination of the LD_{50}, acute dermal toxicity in rats, skin and eye irritation in rabbits, and delayed-type contact sensitization in guinea pigs. Results to date have shown that shale oil and its derivatives are slightly or practically non-toxic, are not lethal if skin exposure is 2 g/kg or less, and are not irritants for skin or eyes. Crude shale oil, hydrotreated shale, and hydrotreated residue were capable of producing sensitization of guinea pigs.

A comparison of these findings with similar studies on materials derived from various coal conversion processes has shown that coal-derived products may have a slightly higher acute toxicity, although they must be labeled slightly to moderately toxic only (LD_{50} between 1 and 10 g/kg).

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INHALATION TOXICOLOGY

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Inhalation Toxicity of Fossil Fuel Related Materials. Inhaled environmental agents related to the use of fossil fuels can significantly alter the production and progression of chronic obstructive pulmonary disease and other disorders. This area has been investigated in three groups of experiments summarized below.

Single high exposures to cadmium aerosol are known to produce lesions in the lung resembling emphysema morphologically. A battery of pulmonary function tests has been used to follow changes with time in the lungs of exposed rats. Alterations were progressive and consistent with the emphysematous lesions observed morphometrically. Adaptation was observed with multiple exposures, but the mechanism is not understood.

The influence of irritant gases on the respiratory tract was investigated with both acute and chronic exposures. Both phospholipid levels and cell proliferation (determined by [^{3}H]thymidine incorporation) increase in the lung after single nitrogen dioxide exposures. The magnitude of these changes and site of thymidine incorporation are being related to the duration of the exposure. Also, vitamin A deficiency was found to increase the phospholipid response without a large change in overall cell proliferation. Vitamin A is necessary for normal epithelial integrity. Chronic daily exposure to nitrogen dioxide produces emphysematous lesions in rats. Under these conditions, the rats are adapted to NO_2, that is, the epithelial necrosis and proliferation resulting from a single exposure is no longer observed. Here, rats were exposed for up to 18 months once per week (to avoid adaptation) to see if similar lesions would result. None were observed morphometrically or by pulmonary function tests. Finally, the observations on the production of a model for bronchitis by sulfur dioxide inhalation were extended biochemically.

A major project is under way on the toxicity of diesel fuel aerosol, used by the military as a visual obscurant. The relative importance of concentration, duration, and frequency of

exposure is being determined in relation to morphology, pulmonary function, alveolar macrophage function, immune function, and neurotoxicity.

Enhancement of Respiratory Tract Tumors In Vivo. Since man is rarely exposed to doses of chemical carcinogens large enough to produce respiratory tract tumors by themselves, studies of environmental factors that may modify the response to carcinogens are of practical importance. Two approaches have been used here. In the first, the "cocarcinogenic" and "promoting" activities of two irritant gases, nitrogen dioxide and formaldehyde, were studied in hamsters. These gases cause necrosis and subsequent proliferation of epithelium in the lower and upper sections of the respiratory tract, respectively. Ten weekly injections of a systemic carcinogen, diethylnitrosamine, were given either before any gas exposures were begun or 2 days after gas exposures (during epithelial proliferation). Gas exposures continued for life. A cocarcinogenic effect was observed in the trachea of animals receiving formaldehyde.

In the second approach, the promotion concept is being investigated in the respiratory tract. A specific region of tracheas of hamsters is lavaged with a solution containing N-methyl-N-nitrosourea to initiate epithelial cells, using a method developed at this laboratory (Yarita and Nettesheim, Int. J. Cancer 22: 298, 1978). The epithelium is subsequently exposed to a known promoting agent on skin, croton oil. Croton oil exposures are by inhalation of an aerosol and continue for life. The morphology of the tracheal epithelium during treatment is being followed. Similar studies are planned with formaldehyde as an enhancing agent.

Ancillary studies on the effect of nitrogen dioxide on pulmonary cell proliferation and phospholipid composition have also been performed.

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POLLUTANTS IN CARDIOVASCULAR DISEASE

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Cadmium and lead are major pollutants associated with solid fossil fuel combustion, coal conversion processes, and solar energy processes. Recent studies have shown that with the increase in demand for solid fossil fuels and solar energy, cadmium and lead pollution of air, water, and soil will be increased by three- to five-fold. These trace elements have been correlated with hypertension and atherosclerosis in epidemiological and clinical studies. In animal studies we have attempted to determine (i) if these trace elements will induce hypertension and/or atherosclerosis, (ii) the optimal concentration required to induce these cardiovascular changes, and (iii) the mechanism responsible for the hypertensive and/or atherosclerotic effect of these trace elements. Results obtained from these studies will

help establish the environmental impact of these energy-related pollutants and afford a means of evaluating the role of cadmium and lead in human hypertension and/or atherosclerosis.

We have observed in rats and pigeons fed (by way of the drinking water) cadmium or lead for 6 to 12 months a sustained increase in both systolic and diastolic blood pressure. In an attempt to resolve the mechanism of cadmium-induced hypertension, we have measured the effects of cadmium on norepinephrine metabolism in vivo. We have observed that cadmium inhibits the uptake of norepinephrine and stimulates the release of this neurotransmitter which results in an increase in plasma norepinephrine. These preliminary observations are of major importance because of the role that norepinephrine plays in regulating blood pressure. Presently we are extending these preliminary observations in various strains of rats. The mechanism responsible for the effects of cadmium and norepinephrine uptake and release is also under investigation.

When White Carneau Pigeons are exposed to drinking water containing 0.8 ppm lead or 0.6 ppm cadmium, they develop atherosclerosis. Morphological assessment of isolated atherosclerotic plaques from lead-treated pigeons show an increase in smooth muscle cells and extracellular lipid droplets, whereas plaques isolated from cadmium-treated pigeons show large numbers of foam cells and extracellular lipid droplets. Similar morphology is observed in human atherosclerotic plaques. In an attempt to expand these in vivo studies we have observed in tissue culture that lead stimulates smooth muscle cell proliferation. The ability of these newly formed cells to metabolize cholesterol and cholesterol esters is presently under investigation. The effects of cadmium on lipoprotein synthesis (in vivo) and lipid metabolism by smooth muscle cells are also under investigation. Results from these studies will provide information necessary for determining the metabolic effects of these trace elements in atherosclerosis.

During the past 3½ years this group has performed experiments designed to gain a better understanding of the mechanism involved in myocardial necrosis. Several experimental models and procedures have been used in these studies. We have observed in the heart that regardless of the method used to induce myocardial necrosis early changes (i.e., within minutes after treatment) include (i) an increase in the size of mitochondria, (ii) a change in the permeability of mitochondria to calcium, and (iii) a change in the permeability of the myocardial cell membrane. Based on reports by Carafoli et al. and results from our group, we suggest that the change in the size of mitochondria is due to the increased mitochondrial uptake of calcium and water. It is furthermore suggested that the change in permeability of the mitochondria and myocardial cell membrane is the result of an increase in membrane lipid peroxidation. Results from this and other laboratories support this latter suggestion. Presently we are attempting to determine if the in vivo results can be reproduced in myocardial cells in culture, which would give us a system for studying the molecular events associated with myocardial cell necrosis. We are also studying the effects of vitamin E and selenium in preventing myocardial cell necrosis.

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CHRONIC TOXICITY OF FOSSIL LIQUIDS

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The objectives of this program are to correlate *in vivo* clinical and pathologic effects with *in vitro* biochemical measures of the mammalian response to complex hydrocarbon mixtures of the type that would occur in liquification or gasification of coal and oil shale.

The work involves empirical bioassay of the chronic local and systemic effects of repetitive dermal application of pure compounds and complex mixtures. These data are used to establish the relative potency of the materials in order to develop biochemical markers of skin exposure at the cellular and molecular level. The question of extrapolation of animal response data to man is being approached by examining the short-term response of human skin in organ culture to agents previously tested on animals to establish the type and degree of biological activity present. By use of the parameters of direct cytotoxicity, cytostasis, rate and characteristics of metabolites generated, and measures of covalent binding of reactive intermediates to critical macromolecules, we hope to obtain a measure of species specific response variation. From this we can better predict the hazard to man represented by a given level of occupational exposure to complex mixtures.

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BIOCHEMICAL DOSIMETRY OF HYDROCARBON MIXTURES

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The objective of this project is to obtain biochemical measures that correlate with the carcinogenic and cytotoxic potentials of various environmental materials, such as products from fossil fuel energy processes. The responsiveness of mouse skin in a short-term organ culture system is being used to screen materials for these characteristics.

Many chemical carcinogens, including benzo[a]pyrene, are metabolized *in vivo* to reactive intermediates that become covalently bound to cellular macromolecules. This binding is a critical event in the process of carcinogenesis.

Our investigations center on the extent and type of covalent interactions between components of hydrocarbon mixtures (particularly of the polycyclic aromatic hydrocarbon type) and the nucleic acids of mouse skin and is approached from two points: (i) the analysis by conventional physio- and biochemical methods (UV-spectroscopy, HPLC, fluorescence, enzymic, etc.) of isolated DNA for adduct formation; and (ii) the study of cellular enzyme systems to determine levels of toxicity and the extent of DNA damage.

Of these, the two methods currently being evaluated are low temperature fluorescence and the induction of polyADP-ribose synthesis. Results to date indicate that each of the two approaches has advantages and disadvantages. Both permit evaluation of pure hydrocarbons or complex mixtures without the use of radiolabeled markers. Fluorescence suffers the disadvantage of limited sensitivity and makes it necessary to use high exposure levels or combine tissue from several animals followed by extensive purification. It is currently assumed that polyADP-ribose synthesis is a marker of early events in repair of subletha' DNA

damage of the type induced by many chemical carcinogens. On this basis it may be possible to correlate polyADP-ribose synthesis with levels of bound metabolite sufficient to induce its synthesis.

Molecular Carcinogenesis

VIROGENE AND ONCOGENE EXPRESSION

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A primary component of this research program is the mechanism of Fv-1 locus restriction studies which are done collaboratively with the group directed by Dr. W. K. Yang and they are described separately.

A second primary component, also in collaboration with Dr. Yang's group, is related to the mechanism of retroviral transformation of hematopoietic cells and it can be divided into three principal approaches: (i) the mechanism of induction of retroviral gene expression or gene transposition by radiation or chemicals, (ii) the mechanism of transformation of specific hematopoietic cell populations by retroviruses and/or retrovirus carcinogen interactions, and (iii) development of methods for detection of transformation of hematopoietic cells in vitro.

We have identified a new class of retrovirus (hydroxyurea and related oxidation products) and have evolved a working model of the mechanism of induction by radiation, halogenated pyrimidines, and other chemicals. The experiments to test this model have indicated that inducers other than halogenated pyrimidines act via one or more types of alteration to cellular or viral DNA sequences, and that cellular repair synthesis can abrogate induction. No specific form of damage has been identified as directly inductive, and it is probable that more than one form of damage may be sufficient. The possibility of a cis-acting reoressor which is a cellular gene function is considered a likely possibility. Studies to further examine this model are in progress. These studies are considered to be important to understanding cellular transformation, since induction of virus gene expression appears to be both necessary and sufficient for transformation, and the ability to develop reagents that specifically detect viral gene products can be useful in determining potential cellular control mechanisms involved in differentiation processes.

The second approach is directed at determining the potential role of endogenous retroviral sequences in the induction of myeloid leukemia in the RFM strain mouse. This strain has been the subject of extensive investigations of radiosensitivity in the Biology Division and circumstantial evidence has implicated retrovirus expression in the etiology of the disease. Extensive analysis of normal and tumor tissue from mice of a variety of ages has thus far provided evidence of only one ecotropic endogenous virus that is inducible or expressed spontaneously. However, further studies have shown that the infection of RFM cells by this virus is restricted in a unique fashion. Our current working model tests the possibility that the restriction of endogenous N-tropic viruses by RFM cells is the result of a third allele of the Fv-1 locus (n') which has been predicted based upon studies by Rowe and

his colleagues. Since only one type of endogenous ecotropic retrovirus is expressed, the probability that tumorigenic recombinant viruses are involved in the neoplastic disease is significantly reduced. Also, the RFM mouse carries a locus that specifically restricts the spread of this ecotropic virus and this suggests a potentially unique mechanism of viral carcinogenesis. Since a variety of evidence suggests that the retroviral genome possesses characteristics which are analogous to bacterial transposition elements (transposons), we are currently attempting to determine whether transposition of RFM virus endogenous sequences can be etiologically linked to any specific radiation induced neoplastic disease in this mouse strain.

Neoplastic disease induced by retroviruses is highly tissue and cell specific, but the limitations of culture technology have not made it possible to study the transformation of cells other than fibroblasts in in vitro systems. Studies now in progress, directly and in collaboration with Dr. Robert Ullrich's group, are directed at the detection of in vitro transformation of specific hematopoietic cells. The approaches involve in vitro exposure to potential carcinogen, extended cultivation and/or implantation into immunologically impaired or at unique sites in susceptible compatible mice. These studies are important, since not only could a successful in vitro transformation system provide a mechanistic approach to target specific carcinogenesis, but also potentially provide a useful model system for studying the alteration of differentiation steps.

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MOLECULAR GENETICS OF CARCINOGENESIS

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The activity of this group has been focused on the molecular mechanism of gene transposition of retroviral genomes in relation to cancer. These genomes are inherent in the germ-line DNA of vertebrates, are capable of moving from one genetic location to another in the cell DNA, and have been found to be associated with neoplastic transformation of the cell in the experimental system. Highlights of our research progress include (i) developing of highly sensitive methods of nucleic acid molecular detection; (ii) finding that host Fv-1 genes restrict the formation of supercoiled DNA duplexes by murine leukemia virus, thus blocking the integration of the viral genome; (iii) elucidating the transposon DNA structure of the DNA genomes of murine N- and B-tropic type C retroviruses; (iv) mapping the chromosome/DNA location of mouse endogenous retroviral genome; and (v) devising an experimental approach for studying retroviral genetic expression in human cancers. Studies are in progress to examine the effect of chemical (e.g., carcinogens) and physical (e.g., irradiation) perturbation of DNA metabolism on transposon behavior of retroviral genomes in the mouse.

Carcinogen-Cell Genome Interaction. The working premise of this research is that the carcinogenic effect (be it due to chemical, physical, or biological agents) involves specific gene elements or specific genetic mechanisms of the cell. Thus, our specific aim has been to identify these gene elements or genetic mechanisms. Our current emphasis is placed on the mechanism of gene transposition and the possible effect of environmental hazardous agents on this genetic mechanism. We are utilizing murine retroviruses as the experimental model, based on the reasons that they are inherent to the germ-line DNA of the mouse, that specific probes for detecting these specific genes are available, that retroviral DNA molecules have sequence characteristics (terminal repeats and inverted repeats) of transposons, and that these gene elements have been shown to have oncogenic potentials in the experimental system.

Highlights of our research progress for this project are as follows.

(i) Cultured human cell lines are highly refractory to infection with murine ecotropic viruses using virion preparations. When inoculated by the transfection procedure with DNA containing genomes of these viruses, human cells showed synthesis of virus specific antigens and also production of progeny virus; however, the expression of the inoculated viral DNA rapidly disappeared after a few *in vitro* passages of the cells, indicating the presence of some controlling mechanism in human cells.

(ii) Employing restriction endonucleases and the agarose gel electrophoresis/diazobenzyloxymethyl-paper transfer/molecular hybridization method, we have analyzed the endogenous retroviral genes in BALB/c mouse cell DNA. In collaboration with Peter Lalley, clones of hamster-mouse cell hybrids are used to assign the chromosome location of individual retroviral genes in the BALB/c mouse. Under the direction of L. R. Boone, a few clones of λ recombinant DNA of BALB/c endogenous retrovirus have been isolated; molecular characterization of these clones is in progress.

(iii) Protein synthesis inhibitors given to the cells in the early hours after retrovirus inoculation can block the retrovirus replication by preventing the formation of supercoiled viral DNAs. This finding suggests that integration of retroviral genome into cellular DNA requires the function of certain proteins synthesized early in the infection (6).

(iv) Genetic expression of retrovirus genes is strongly affected by the differentiation state of the cell. Undifferentiated murine teratocarcinoma cells are highly refractory to infection with ecotropic retroviruses, although the synthesis of both linear and supercoiled full genome-length retroviral DNAs appears to be unaltered (3). Thus, undifferentiated teratocarcinoma cells may be useful for studying the control of the gene integration process or postintegration steps of retroviral gene expression.

Mechanism of Fv-1 Gene Restriction. Fv-1 locus, an autosomal dominant gene located in chromosome 4 of the mouse, has been shown to be an important host factor for the control of horizontal spread of murine leukemia viruses during viral leukemogenesis in the animal as well as in the infection of cultured mouse cells in vitro. Cells with Fv-1^n alleles are resistant to B-tropic viruses and susceptible to N-tropic viruses, whereas cells with Fv-1^b alleles are resistant to N-tropic viruses and are susceptible to B-tropic viruses. The aim of this research project has been to elucidate the restriction mechanism at the molecular level. The research has involved the collaborative efforts of R. W. Tennant's and W. K. Yang's groups at ORNL, A. Brown at the University of Tennessee-Knoxville, and R. H. Bassin at the National Cancer Institute.

In previous studies we demonstrated that the Fv-1 resistance property against the infection of N- and B-tropic viruses could be transferred to the susceptible cells by means of cell free extract (Proc. Natl. Acad. Sci. 71: 4241–4245, 1974), that the resistance transfer activities of the cell-free extracts segregated specifically with the Fv-1 alleles in mouse genetic cross experiments (J. Virol. 20: 589–596, 1976), and that active components of the cell-free extracts were distinct polyA RNA molecules. These studies indicate that the Fv-1 restriction is mediated by specific RNA products present in the cytoplasm of the cell and, hence, suggest that the restrictive action is the result of direct interaction between the Fv-1 gene product and virus target molecules, whose presence in the virion particles has been indicated by studies of other investigators. Continued biological characterization was performed (1,2).

Recently, we have placed our efforts mainly to elucidate the precise step of Fv-1 restriction in the replication cycle of these leukemia viruses. Procedures of DNA transfection suitable for studying Fv-1 restrictive and permissive mouse cell cultures have been developed (Cancer Res. 37: 1709–1714, 1977). With these procedures, we have found that transfection with integrated virus DNA of N- and B-tropic viruses, unlike infection with virion preparations, is not affected by Fv-1 alleles of the cell. Further, by the DNA transfection assay, the unintegrated viral DNA preparations isolated from Fv-1 restricted infection were found to be much less infectious than those from Fv-1 permissive infection. These results suggested that Fv-1 restriction occurs at the step of reverse transcription and causes a defective viral DNA synthesis (1).

To further study the precise molecular defect, a sensitive method has been established, that allows quantitation of viral DNA duplexes in cells infected with retroviruses at m.o.i.'s of 0.01 and above. Using this method, we have found two types of Fv-1 restriction on viral DNA synthesis. In one type of restriction, which was present in all Fv-1^b cells examined and a few Fv-1^n cells, conversion of viral linear DNA duplex (form III) to co-

valently closed circular supercoiled DNAs (form I) is blocked (5). In the other types of restriction, which are present in many Fv-1^n cells (notably RFM mouse cells), formation of viral DNA duplex is blocked and hence all full genome-length viral DNA intermediates are severely depressed (5). In the first type of restriction, when Fv-1 restriction is abrogated with heat-inactivated virus of the same tropism, production of form I DNAs is restored. Preliminary results suggest that the Fv-1 caused defect might be present in the terminally repeated sequences of viral linear DNA duplex of the restricted virus.

In vitro synthesis of full genome-length viral DNA duplex has been possible with N- and B-tropic viruses in this laboratory. Effect of Fv-1 cell extract on the in vitro reverse transcription is being examined.

Retroviral Genetic Expression in Human Cancers: Analysis by Primer tRNA Binding Approach. The particular experimental approach that forms the basis of this research project is to use the property of "primer-tRNA" binding as a marker to identify the presumed retrovirus-related specific RNA molecules in human cancer cells (4). It is well established that reverse transcription of retroviral genome is initiated on a primer, which is a tRNA molecule of cellular origin (e.g., our previous work, Proc. Natl. Acad. Sci. 72: 2155–2159, 1975). All retroviruses examined have been found to utilize a tRNA as the primer and contain a primer tRNA-binding sequence in their RNA genome. The tRNA-binding sequence is located in the "leader" region of virus-specific mRNA; it is an essential structure for the synthesis of large terminal repeats in the retroviral DNA. However, we are keenly aware of the current widespread skepticism among cancer researchers concerning the relationship between retroviruses and human cancers and the need for producing unambiguous research data in this regard. Also, the number of copies of intracellular primer-binding polyA RNA molecules may be small; an extraordinarily sensitive method is required for specific demonstration of these molecules.

A pilot study comparing a human fibrosarcoma cultured line (HT1080), a normal foreskin-derived fibroblast line (HS8P), a breast cancer line (ALAB), a rhabdomyosarcoma line (A204), and a bronchogenic carcinoma line (A549) showed promising preliminary results of the tRNA binding approach but also indicated technical complications and difficulties, such as requirement of large quantities of cell materials and contamination of ribosomal RNAs in the polyA preparations. Hence, we have adapted the diazobenzyloxymethyl-paper transfer procedure of Alwine/Wahl/Stark for the purpose of this study and found it to be highly satisfactory. Continued studies are in progress by employing this highly sensitive RNA detection method.

In this study we have found that cellular ribosomal RNA, as well as genomic RNA of retroviruses, is able to bind selective tRNA species in an in vitro molecular hybridization reaction and, at least in the cases of chicken and mouse, the binding appears to involve the same tRNA molecules (4). Human ribosomal RNAs selectively bind two out of the four glutamic acid tRNA isoacceptors. The in vitro formed rRNA.tRNA complexes are able to serve as template-primer for DNA synthesis by reverse transcriptase. "Strong-stop" type of DNA products was observed. DNA synthesis from chicken 28S rRNA was about 150 base long but its nucleotide sequence structure was distinctly different from that of the strong-stop DNA of avian myeloblastosis virus. Employing a cloned DNA of mouse ribosomal DNA gene and restriction endonuclease mapping, we located the binding site for proline tRNA to be at the 3' portion of 28S ribosomal RNA.

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REGULATION OF GENE EXPRESSION

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The objectives of this research are to define in molecular terms the mechanisms determining expression of specific genes in mammalian cells, how these are regulated by hormones and other specific effectors, and the events responsible for the dysfunction of gene expression in cells transformed to malignancy. Much of our work is focused on expression of the rat liver enzyme tyrosine aminotransferase (TAT), a convenient model since expression can be experimentally manipulated in a number of important ways and the system is amenable to detailed biochemical analysis. In recent work, progress has been made in several experimental approaches.

Preparation of cDNA^{TAT}. Production of this indispensable reagent has been our principal tactical goal for some time, but progress has been slow owing to the very small quantities of mRNA^{TAT} present in liver cells (ca. 0.03% of total mRNA). We have now worked out rapid isolation and purification methods to enrich mRNA^{TAT} to 5 – 10% purity; translation assay indicates that this is the principal mRNA component of such preparations. Enzymically synthesized cDNAs have been purified in two ways: (i) repetitive low Rot hybridizations, which yield a cDNA which is kinetically homogeneous and has the expected characteristics of cDNA^{TAT}, but in very small quantity; (ii) molecular cloning, wherein the partially purified cDNAs were inserted into the Pst-1 site of plasmid pBR322. After antibiotic selections, 40 clones, determined by gel electrophoresis to contain inserts of significant size, were selected for study. At least one of these appears to contain cDNA^{TAT}, an insert of ca. 850 bp that is removed intact by Pst-1 cleavage and selectively hybridizes to an mRNA that codes for protein reactive with anti-TAT antibody. However, difficulties are being encountered in obtaining a completely definitive result in this hybridization-elution-translation assay, the ultimate and necessary proof of cDNA identity. Another of the clones studied has been provisionally identified as carrying a cDNA for tryptophan dioxygenase, a third for albumin, etc. This approach will thus permit development of a "library" of cloned cDNAs specific for the gene products of rat liver; such a library will be of great value in analyses of various aspects of regulation of gene expression.

Hormonal Control of Gene Expression. Synthesis of TAT is induced by glucocorticoids, cAMP, and insulin, the steroid- and cAMP-type inductions being highly specific while insulin appears to promote a generalized increase in synthesis of liver proteins which is especially effective for TAT. In earlier work we presented data suggesting that the steroid induction involved acceleration of production of $mRNA^{TAT}$, while insulin and cAMP appeared to promote translation of existing mRNAs. Polysomal mRNAs from livers of control and hormone-treated rats were analyzed by translation in reticulocyte lysates. As expected, hydrocortisone increased $mRNA^{TAT}$ 8- to 10-fold in accord with all earlier data and with analyses by others of the mechanism of action of steroid hormones. Analyses of mRNAs after insulin and cAMP treatment yielded the surprising result that $mRNA^{TAT}$ activity is increased by these effectors as well and to an extent consistent with the increased enzyme synthesis. Since the translation assay measures biological activity of mRNAs, which may or may not be equivalent to quantity, a definitive interpretation of these results requires chemical mRNA assays. These have been initiated, quantitating $mRNA^{TAT}$ sequences with $cDNA^{TAT}$ purified by hybridization. Preliminary results indicate: (i) that hydrocortisone increases $mRNA^{TAT}$ sequences, again as anticipated; (ii) that neither insulin nor cAMP effect significant increases in $mRNA^{TAT}$ sequences. The latter result, if confirmed, points to the existence of hitherto unsuspected control mechanisms involving alterations in mRNA structure which improve its translational efficiency. Since inductions by cAMP and by insulin are known to be mechanistically different, it appears that two such mechanisms are operative in hormonal control of gene expression.

Enzymic Differentiation. Levels of TAT are very low and insensitive to steroids prior to birth; within a few hours after birth or if fetal livers are placed in organ culture, enzyme levels rise and become inducible. These and other observations suggest that TAT expression is suppressed until removal from the intrauterine environment permits a rapid differentiation, with the gene transiting from essentially silent to fully active and sensitive to hormonal modulation very quickly. Analyses by translation in reticulocyte lysates have been made of functional $mRNA^{TAT}$ levels in polysomal mRNA preparations from livers of rats in the perinatal period. Results are in accord with this postulate. In fetal livers or those from newborns 0-2 h after birth, $mRNA^{TAT}$ levels are below the limit of detection in this rather insensitive assay, and hydrocortisone does not increase these levels in treated fetuses. The mRNA becomes detectable at about 3 h and thereafter rises quickly in accord with changes of the enzyme. Once the mRNA has appeared it can be increased by glucocorticoids, as in the adult.

Gene Expression in Malignant Cells. Several cultured hepatocellular carcinomas in which TAT expression has been reported to be altered were examined. We confirmed the absence of TAT enzymic activity in most of these and found that the low apparent TAT activity of others (e.g. IAR, K-16) is actually that of another aminotransferase capable of utilizing tyrosine as amino donor. Translation assay revealed that none of these contain detectable levels of $mRNA^{TAT}$. Thus the structural gene appears to have been silenced (or perhaps lost) in the transition to malignancy. In other work we have found that the metallocarcinogen beryllium selectively inhibits induction of TAT by glucocorticoids in H-35 cells; control of expression by insulin or cAMP was not affected.

Turnover of Gene Products. Previous work has indicated that TAT turnover is mechanistically unusual, perhaps involving participation of a specific cellular component which may be a polypeptide. Screening of a number of strains of mice from the Division Mouse Genetics

facility for a slow-turnover strain (perhaps mutant in the putative factor) did not succeed; those strains with unusually high TAT levels synthesized the enzyme faster but did not differ in K_D. Another approach involves analysis of the disappearance of endogenous or added TAT in lysates of H-35 cells, a process resembling in many ways the intracellular turnover of the enzyme. The loss of enzymic and immunological activity of ^{3}H-labeled TAT is associated with its conversion to a form which chromatographs in the void volume on gel filtration; when analyzed on glycerol gradients, this apparent gain in molecular weight appears to be a biphasic process. Analysis of the high molecular weight form on NaDodSO$_4$-PAGE showed that the 50,000 dalton subunit of the enzyme was essentially intact; thus neither covalent interaction with another macromolecule nor extensive proteolysis had occurred. In a third approach we assessed the effect of concanavalin A, reported by Thompson et al. (J. Biol. Chem. 252: 2717–2725, 1977), to effect a rapid and reversible reduction in TAT levels of cultured hepatoma cells. We found (i) that the loss of catalytic activity and of reactivity with antibody to native TAT is associated with appearance of a form reactive with antibody to TAT subunits, and (ii) that this form does not undergo the rapid metabolic turnover of active, dimeric TAT. As yet the significance of these puzzling observations is not apparent.

Turnover of mRNA^{TAT}, like that of the enzyme, is unusually rapid. We have measured the rate of synthesis and functional mRNA levels of alanine aminotransferase (AAT), a typical gene product of the liver in that turnover rates of both enzyme and mRNA are those of the bulk of these macromolecules. Synthesis of AAT is roughly 10% of that of TAT, owing to a comparable reduction in levels of mRNA^{AAT} present in steady-state conditions. When the turnover rates are taken into account, it appears that transcription of the TAT gene is 100 to 300 times more active than that of the average gene of rat liver. If verified, this difference is expected to be most advantageous in future probes of gene expression with this model system.

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Normal and Malignant Cell Biology

TUMOR CELL SURFACE PROTEINS

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Cell surface proteins mediate interaction between cells and their environment. Unique tumor cell surface proteins are being identified and quantitated in several tumor systems to address the following questions: (i) How do tumor-specific proteins arise during cell

transformation? (ii) Can these proteins be used as markers of tumor cell distribution *in vivo*? (iii) Can cytotoxic drugs be targeted specifically to tumor cells using antibody (iv) Can solid state radioimmunoassay of these proteins provide a means to quantitate transformation frequencies?

A tumor surface protein of 180,000 M_r (TSP-180) has been identified on several lung carcinomas of BALB/c mice. TSP-180 was not detected on normal lung tissue, embryonic tissue, or other epithelial or sarcoma tumors, but it was found on lung carcinomas of other strains of mice. Considerable amino acid sequence homology exists among TSP-180's from several cell sources, indicating that TSP-180 synthesis is directed by normal cellular genes although it is not expressed in normal cells. The regulation of synthesis of TSP-180 and its relationship to normal cell surface proteins is being studied.

Proteins such as TSP-180, the *src* gene product, and plasminogen activator are expressed on certain tumor cells but not normal cells. Antibody reagents reacting specifically with these tumor antigens should "home" to target cells *in vivo*. Using radiolabeled antibody, tumor cells and their metastases can be located by radioimaging. Pure antibody reagents needed for these studies can be obtained using the technique of hybridoma formation. Hybridomas producing monoclonal antibody to lung tumor cell surface determinants are under test for specificity and avidity of reaction. Production of large amounts of appropriate monoclonal antibodies should facilitate radioimaging experiments.

If antibodies can localize at tumor cell sites, they should be useful for delivery of cytotoxic chemicals to the tumor cells. Intermediate carriers such as dextran and liposomes should allow large numbers of drugs to be delivered by a limited number of antibody molecules. Monoclonal antibodies have been covalently derivitized with fatty acids and attached to liposomes. These liposomes bind specifically to target cells *in vitro* but show very little increase in efficiency of drug delivery relative to free drug. Methods to promote more efficient drug transfer from liposomes to tumor cells and specific liposome targeting *in vivo* are now being tried. This specific chemotherapy should be useful as adjuvant therapy to surgery or radiation of primary tumors.

Finally, tumor-specific proteins represent cell phenotypes characteristic of cell transformation. Quantitation and sensitive detection of these proteins should provide a method of early detection of transformation. Automated solid state radioimmunoassay techniques have been developed that can be used to screen thousands of cell clones for transformed phenotypes. Assays using monoclonal antibody coupled to solid supports should allow assay of more than 10^6 cells at a time. Development of these techniques and their use to screen carcinogen treated cells is under way.

Study of Antigenic Markers in Developing Neoplasia. Most malignant tumors of man arise from epithelia via a multistep process. However, only recently have there been reports that describe such transformational differentiation *in vitro*. Rat tracheal epithelial cells that have been treated with carcinogens progress from preneoplastic to tumorigenic cell populations in tissue culture. It is of interest to determine if such *in vitro* transformed epithelial cells also bear unique antigenic markers and, more importantly, when during carcinogenesis these antigens appear.

During the past year we have investigated whether late passaged tumorigenic tracheal epithelial carcinomas induced by *N*-methyl-*N*'-nitro-*N*-nitrosguanidine are antigenic in immunocompetent syngeneic animals. Only after repeated challenges was there evidence of transplantation resistance. Once transplantation-resistance was established, both humoral

and cell-mediated immune responses were readily evident. No response was detected to normal primary epithelial cells which are the presumed target cells for *in vitro* transformation. Cultures of preneoplastic and neoplastic cells of the same lineage are being tested quantitatively for expression of these antigens to probe the mechanism of neoplastic transformation *in vitro*.

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CHEMICAL EFFECTS ON THE IMMUNE SYSTEM

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The purpose of this research is to explore the effects of selected energy-related and nonenergy-related chemicals on immunity and host defense mechanisms. Two primary areas are under investigation. One area is mechanistic studies aimed primarily toward understanding the role of the immune system in chemical carcinogenesis. Diethylnitrosamine carcinogenesis in mice of diverse immunologic constitutions has shown disproportionate correlations between tumor induction of different tumors in different organs and changes in immune competence emphasizing that observations on only one type of tumor can be very misleading and that both immunologic and nonimmunologic factors contribute. Therefore, different tumor cell lines and clones from diethylnitrosamine-induced carcinomas are being characterized at different *in vitro* and *in vivo* passage times to compare changes in immunogenicity, tumor growth rate, latency, TD_{50}, and metastatic capacities. Cross-reactivity among these tumor lines is also being determined. Our objective is to examine the various cell lines and the sera from tumor-bearing hosts in an effort to characterize and define specific roles and mechanisms as they relate to both immunologic and nonimmunologic parameters. The second area is the establishment of the technology to evaluate the activity of suspect chemicals with regard to immunologic toxicity and to obtain fundamental information on the immunodestructive effects and reversibility or recovery from such insults, i.e., the acute and chronic effects of such injury at or near environmental levels, and to further determine an order of magnitude for significant immunosuppressive activity, successful recovery and/or subsequent late effects.

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THE PROCESSES AND REGULATION OF MEGAKARYOCYTOPOIESIS AND PLATELET PRODUCTION

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The general objectives of this research program are to elucidate the maturation processes and the regulation of the megakaryocyte-platelet axis of the hematopoietic system in order to provide a basis for understanding, manipulating, and alleviating the deleterious health effects of environmental agents associated with various energy-producing processes. Since the rate of turnover of the circulating, functional cells of the hematopoietic system and their precursors in the blood-forming tissues is among the most rapid in mammalian organisms, changes in various physiologic and environmental factors can result in early, damaging effects on the host organism. It is therefore important to understand megakaryocytopoiesis and platelet production and how they are regulated in order to prevent, circumvent, or treat damage. In addition, such information will be valuable in establishing methods for assessing toxicologic damage introduced by exposure to potentially hazardous chemicals and metals.

Injection of plasma from platelet-poor donor rats resulted in an increase in the endomitotic index of megakaryocytes of recipient mice 32 h after the initial treatment with plasma. The results suggested a dose-response relationship between the amount of plasma administered and the degree of stimulation of megakaryocytopoiesis. These findings demonstrate that an agent capable of stimulating megakaryocytopoiesis is released in response to thrombocytopenia and that this factor can be successfully transferred between species. They also substantiate the assumption that the increase in peripheral platelet numbers and in platelet labeling after administration of presumptive thrombopoietin occurs via stimulation of megakaryocytopoiesis.

In another set of experiments, rats were exchange transfused with platelet-poor blood to produce varying degrees of thrombocytopenia. Marrow samples were taken 31 h later and the megakaryocyte population was scored for the percentage in endomitosis. The endomitotic index of megakaryocytes progressively increased as the circulating platelet count was reduced. These findings are consistent with the concept of a humoral regulator of thrombocytopoiesis, the activity of which is related to the severity of depression of circulating platelets.

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IMPROVEMENT IN RECOMBINANT DNA TECHNOLOGY

G. D. Novelli S. I. Simms
A. N. Best

Background. In 1971 reversed phase column chromatography (RPC-5) for the separation of individual amino acid-specific transfer ribonucleic acids was invented at ORNL in the Macromolecular Separations Technology Program. In 1976 R. D. Wells at the University of Wisconsin accidentally discovered that RPC-5 chromatography has superior capability of separating double-stranded fragments of DNA (called recombinant DNA). The solid support for RPC-5 columns is polychlorotrifluoroethylene, Plaskon CTFE 2300 powder made by Allied Chemical Company. Unfortunately in 1975 Allied Chemical Company stopped producing Plaskon CTFE 2300 (it was a by-product of another process). This then made us the sole possessors of this valuable resource. As a consequence of Well's publication we became inundated with requests for this material by molecular biologists from all over the world who are interested in doing gene splicing and recombinant DNA experiments. Because of the foregoing circumstances, we felt obligated to seek a suitable substitute for Plaskon CTFE 2300 and to study the operating parameters for the improved chromatography of the restriction fragments.

Specific Aims and Scope. (i) To seek out similar, but commercially available, solid support materials as a substitute for the no longer available Plaskon CTFE 2300. (ii) To study the operating parameters of the RPC-5 system for improved and efficient separation of DNA restriction fragments.

Progress to Date. We have tested six samples of polytetrafluorethylenes, Kel-F and lichromosorbs of differing particle size, using Escherichia coli tRNA as the feedstock (since most of our experience has been with such material). So far we have not found a matrix that even approaches Plaskon with respect to flow rate and separation efficiency.

In the second and related part of this program, we have successfully applied RPC-5 chromatography to isolate in good yield the superhelical plasmid DNA from E. coli. This is the substance that is the starting material to produce restriction DNA fragments for further genetic engineering experiments and is conventionally prepared by a tedious series of ultracentrifugation runs in cesium chloride gradients that yield small amounts of material. The advantages of our RPC-5 procedure are that it is much less costly, much more rapid, and can be scaled up to produce larger quantities of starting materials.

IMPROVED PROCEDURES FOR THE LARGE SCALE CULTIVATION OF MICROORGANISMS AND MAMMALIAN CELL CULTURES AND PRODUCTS THEREOF

G. D. Novelli E. F. Phares
M. V. Long

Since each microorganism, cell culture, or products therefrom present a new challenge, each project we undertake represents a new research project. Normally we initiate a new project on a relatively modest scale (20-30 liters) and proceed to evaluate the medium constituents, pH, temperature, operating speed, rate of aeration, etc. to produce the maximum yield of the desired product with the greatest efficiency in time and smoothness of operation. Having established the optimal conditions that will yield the best product at the lowest cost in time and money, the process is scaled up to produce the needed amount of material.

We have carried out collaborative work with about 15 groups in the Biology Division, three other divisions at ORNL, seven universities, and the National Institutes of Health that had special problems that could only be carried out in this facility.

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Interdivision Activities

Life Sciences Synthetic Fuels Program

Coordinators	- J. L. Epler - R. J. M. Fry
Microbial Systems	- T. K. Rao - F. W. Larimer
Cytotoxicity and Embryo Toxicity	- J. N. Dumont - T. W. Schultz
Repair	- J. D. Regan
Mammalian Cells	- A. W. Hsie
Teratogenesis	- L. B. Russell - R. Filler
Mammalian Mutagenesis	- L. B. Russell - W. M. Generoso
Toxicology	- H. Witschi - L. H. Smith - W. M. Haschek
Neurotoxicity	- A. P. Pfuderer
Carcinogenesis	- J. M. Holland

A multi-divisional effort aimed at the integrated assessment of the health and environmental effects of various coal conversion and shale oil technologies is being carried out. The Biology Division's efforts are summarized below.

GASIFIERS IN INDUSTRY

Statement of Problem. The principal objective of this project is to test the primary effluents and fugitive emissions from low Btu gasifier processes for their potential effects on man. The specific issues include (i) potential effects on man of process discharges to air or water, (ii) exploration of environmentally acceptable methods for disposal of solid waste produced from small low Btu gasifiers, and (iii) determination of hazards associated with the handling and combustion of products from gasifiers.

Research Approach. Our approach to answering these questions, in an expeditious and cost-effective manner, involves a parallel, two-level program. Level one is cellular bioassays. These assays provide baseline data on typical effluents and emissions and ascertain how the relative toxicity of major effluents and fractions thereof vary as a function of changes in process conditions. In addition, biological effects studies using cellular assays will provide an essential data base for eventual determination of correlations with whole animal studies.

Level two consists of the mammalian toxicity and carcinogenicity bioassays. As data become available through analytical chemistry, area monitoring, and cellular bioassay programs, they can be used in guiding decisions concerning whether other materials should be tested and whether additional assays should be carried out in mammals.

With short-term mutagenicity assays, testing with only one microbial system has been shown to be faulty in a number of cases with pure compounds. We propose to improve the reliability of information by using a battery of short-term genetic and/or DNA assays on the primary effluents and potential fugitive emissions. Thus, potential hazards from both the mutagenic and carcinogenic aspects will be more carefully "screened." Furthermore, some segments of the battery of assays are being used only on selected compounds determined by the coupled effort of chemical separation and analysis and initial biological screens. The actual components can then be characterized as either highly purified fractions or actual pure chemicals. Feedback to chemical screening then becomes a feasible monitoring method.

The battery of tests is composed of the following: (i) Salmonella histidine reversion (Ames test), (ii) Mammalian cell and/or yeast gene mutation and recombination/conversion assays, (iii) DNA repair, and (iv) in vitro cell transformation. Only selected samples will be tested in the full battery of assays, depending largely on the preliminary results of the Ames screening and the chemical analyses. Cytotoxicity will be considered a normal component of the genetic/DNA screens; however, other assays that look rapidly and specifically at toxic effects on "cellular" systems will also be carried out with appropriate samples and selected active compounds.

The search for heritable genetic changes in mammalian germ cells is the most critical and definitive test for the assessment of genetic risk to human beings. Samples to be tested here must be carefully chosen and considerable progressive screening carried out before general application. The choice of samples to evaluate can be modified or number of samples increased in relation to the results of the cellular bioassays and/or chemical screening.

Five types of tests will be considered in the mouse system, two of which detect chromosomal damage and one detects the induction of point mutations. The fourth genetic test, the rapid so-called "spot test," probably also detects point mutations but in somatic cells. The fifth mouse test is designed to be a sensitive indicator of germ cell killing by mutagens and of possible effects on reproductive physiology.

Initially it is planned to include only dominant lethals and the spot test of the five mammalian tests in the comparative assessment of genetic risk from the low-Btu gasifier. The other assays (also including DNA repair systems) are currently being used in the generic approach with other fossil energy-related compounds and would interrelate with and reinforce the site-specific evaluations.

Information gained in the preceding integrated program should provide the assessor with specific information on specific process materials. The generic approach, coupled with the chemistry, health effects studies, and environmental studies, should place these materials in context with respect to the data base currently available. Information on the potential mutagenicity, carcinogenicity, and overall toxicity of the multiply tested materials can be placed in perspective with results from other gasifiers and other technologies. Comparative information and the published data on similar materials should permit some ordered estimate of biohazard on each unit.

Progress. Two coal gasification sites are under test or under construction at the University of Minnesota, Duluth, and Pike County, Kentucky. Comparative work on ESP tar samples obtained thus far has shown mutagenic activity in materials from both sites. Activity (as measured by the Ames test) appears to be concentrated in the PAH fraction(s).

Selected fractions from the tar samples are being assayed in the yeast mutagenesis system and are scheduled for mammalian cell mutagenesis testing. Preliminary work is also under way in the whole animal (mouse) mutagenesis system.

Acute mammalian toxicology studies were completed with materials sampled from three ports. Given orally, the materials were slightly toxic to mice. No acute skin toxicity was found in rats, and tests for skin irritation and delayed type hypersensitivity were negative. However, all three test materials were moderately irritating to the eye, producing conjunctival edema and injection and corneal lesions. Seven days after the tests, the eye lesions had healed. The skin carcinogenesis assays are being carried out on samples of the liquid waste stream. Although the experiment is not complete, there are indications that components of the waste streams are carcinogenic in mouse skin.

H-COAL LIQUEFACTION

Statement of Problem. The feasibility of using short-term assays to predict the potential biohazard of various shale oil and H-Coal test materials will be examined in a coupled chemical and biological approach. The primary focus of the research will be the use of preliminary chemical characterizations and preparation for bioassay, followed by testing in short-term assays in order to rapidly ascertain the biohazard.

Research Approach. Using crude and/or fractionated materials, simple bioassay systems are being used to determine which materials or fractions thereof are biologically active, thus aiding in the assignment of priorities for further chemical separation and characterization. Additionally, secondary screening of partially defined constituents aids in determining which mixtures, classes, or specific compounds require testing in intact animal or plant systems. Conversely, complex materials that are known or prove to be active in higher organisms can be assessed with the short-term tests and, again, detailed chemical analyses can be guided by the display of biological (genetic) activity. The overall approach may validate the use of short-term genetic screening systems to predict mutagenicity and carcinogenicity for intact organisms and man. Implied in the coupled chemical-biological approach is the application of and further development of bioassays, not only for detecting hazardous materials in environmental effluents and process streams but also for measuring and monitoring these materials, via bioassays in the general environment, in the work place, and during their storage (or disposal) and transport. Furthermore, methods for applying short-term tests to the monitoring of exposed individuals through cytogenetic assays or microbial screening assays utilizing body fluids are under development. Preliminary information concerning the metabolic mechanisms of activation, the definition of cellular and molecular mechanisms of damage, and the repair of damage caused by key compounds (from the major classes of chemical pollutants) is being accumulated along with the determination of potential genetic biohazard.

The H-Coal program will be carried out in two phases. Phase I will assess samples that are currently available from pilot demonstration scale operations; short-term mutagenesis, cytotoxicity along with mammalian toxicity, and skin carcinogenesis assays will be carried

out with these materials. Phase II will assess samples developed when the H-Coal plant is under way. Phase I tests (already under way) will include the H-Coal raw distillate, various stages of upgrading, and H-Coal products. The preliminary assays will parallel existing efforts with other syncrudes. The information received should aid in selection of actual process samples for evaluating the Catlettsburg, Kentucky, H-Coal pilot plant now under construction. It will also provide useful comparison of the changes which occur in the biological characteristics of specific process liquids as a function of scale-up.

Information gained in the preceding integrated program will provide the assessor with specific information on specific process materials. The generic approach, coupled with the chemistry, health effects studies, and environmental studies, should place these materials in context with respect to the data base currently available. Direct information on the potential mutagenicity, carcinogenicity, and overall toxicity of the materials tested can be placed in perspective with results from other technologies. Comparative information and the published data on similar materials should permit some ordered estimate of the biohazards of each plant.

Progress. All short-term bacterial determinations within Phase I of H-Coal have been completed. A reduction in mutagenic activity parallels the heavy distillate (aromatic fractions). Cytotoxicity work can be summarized in a similar manner: toxicity is reduced with hydrotreatment.

Evaluations with the Ames assay of the crude shale oils versus hydrotreated oils reinforce our work with synthetic fuels from liquefaction, i.e., a reduction of activity after hydrotreatment. Cytotoxicity work parallels this observation.

Selected fractions are under test in comparative short-term systems. Choice of samples (and/or fractions thereof) to be used for validative testing will depend on both the preliminary biological work and the chemistry. The validation (extension to higher organisms) will include tests for mutagenesis, e. g., mammalian cell gene mutation, whole-mammal mutation (mouse) and Drosophila for cytogenetic damage, e.g., sister chromatid exchange.

Mammalian toxicity assays showed that a variety of distillates and oils had slight to moderate toxicity when given orally to mice. Reversible eye irritation was found with hydrotreated light organic liquid, SRC wash solvent and SRC fuel oil blend, but not with hydroprocessed coal distillate and SRC recycle solvent. Samples from the process streams have been found to be potent mouse skin carcinogens. The relative potency of these complexes is being determined currently. The present data suggest that the addition of hydrogen (hydrotreatment) also decreases the carcinogenicity but does not reduce some of the systemic toxic effects. Hydroprocessed distillates absorbed through the skin have caused effects on the kidney either directly or by an abscopal effect.

PARAHO/SOHIO SHALE OIL

The principal focus of the Paraho/SOHIO Shale Oil project is the testing of primary effluents and products for potential effects on man. This portion of the evaluation of Paraho samples is concerned with questions of relative toxicities of process materials and refinery products.

The design of our approach to answer these questions in an expeditious and cost-effective manner involves a parallel, two-level program. Level one is cellular bioassays. These

assays will accumulate baseline data on typical effluents and emissions and ascertain how the relative toxicity of major effluents and fractions thereof vary as a function of changes in process conditions. In addition, biological-effects studies using cellular assays will provide an essential data base for eventual determination of correlation with whole animal, acute, and chronic toxic effects.

Level two consists of the mammalian toxicity bioassays. These assays involve characterization of the acute, subacute, and chronic toxicity of primary process precursors and products. A study on acute mammalian toxicity of selected Paraho samples was completed. Overall oral and dermal toxicity of shale oil and some of its derivatives was comparable to petroleum-derived products: all samples were only slightly or partially nontoxic and were not irritants for skin or eyes. However, crude shale oil, hydrotreated shale oil, and hydrotreated residue produced delayed type contact sensitization in guinea pigs. Preliminary data with the mouse lung adenoma assay suggest that crude shale oil has carcinogenic potential. Skin carcinogenicity has been obtained, in order of potency, for the crude shale oil, the residue after hydrotreatment and the hydrotreated shale oil. Systemic toxicity involving damage to the distal portion of the renal nephron has been noted 8 months after exposure to distillates from both shale oil and natural petroleum.

The Gene-Tox Workshop Task

W. Winton, Coordinator
J. L. Epler, Scientific Adviser
in collaboration with the
Environmental Mutagen Information Center

Under an interagency agreement with the U.S. Environmental Protection Agency, Biology Division staff have worked with the Environmental Mutagen Information Center of the Information Division to set up a series of workshops to evaluate the current status of bioassays on genetic toxicology.

The Office of Pesticides and Toxic Substances of the U.S. Environmental Protection Agency needs evaluations of the current status of bioassays that determine the mutagenesis and presumptive carcinogenesis of chemical substances. To achieve this objective, a series of workshops on genetic toxicology have been held in which prominent research scientists from academic, industrial, and governmental centers have participated.

Members of the Biology Division staff coordinated the workshops and acted as scientific advisers to the project. Furthermore, W. M. Generoso, A. W. Hsie, E. Huberman, R. J. Preston, and L. B. Russell of the Biology Division acted as workshop leaders for the project.

Education Activities

University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences

The University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences is located within the Biology Division of Oak Ridge National Laboratory. The program is primarily designed for training leading to the Ph.D. degree although there are a few Master's degree candidates. Ph.D. students are supported by The University of Tennessee in the form of research assistanships or by one of the four federal training grants awarded to the School by (i) the National Cancer Institute, (ii) the National Institutes of Health in the form of a training grant in genetics, (iii) the National Institutes of Health in the form of a training grant in cellular and macromolecular sciences, and (iv) the National Institute of Aging. The School currently has 41 students working toward the Ph.D. degree and 7 in the Master's program. As of June 1980, 68 students have been awarded the Ph.D. degree.

The fall quarter of 1980 marks the beginning of the School's fifteenth year. W. Edgar Barnett and R. Julian Preston are the Director and Associate Director, respectively. The School also has four full-time faculty members: Daniel Billen, Frank H. Gaertner, Donald E. Olins, and Nathaniel W. Revis. A major portion of the school's teaching and research training is provided by the staff of the Biology Division who serve as "shared" faculty.

The students form a very active group of investigators in training, and their names appear on a number of manuscripts each year. This represents a significant contribution to the productivity and excellence of ORNL's Biology Division.

Postdoctoral Training Programs

Postdoctoral training is an important feature of Division activities, and the training programs are of mutual benefit to the trainees and the Division. Support for these training activities is derived from several sources. The following table lists the funding sources and the number of trainees presently supported by each one.

Source	No. of Trainees
National Cancer Institute Carcinogenesis Training Grant*	5
National Institute of Aging Aging Training Grant*	3
Biology Division Postdoctoral Investigatorship	17
Research programs of Biology staff members	7
National Cancer Institute	1
Monsanto Toxicology Fund	1
Total	34

*Grant made to the University of Tennessee in response to proposal submitted by Division staff members.

Subcontract between the Biology Division and the University of Tennessee.

After a two- to three-year period of research in the Biology Division, past trainees have obtained positions in universities, industries, and government laboratories.

Undergraduate Training Programs

The Biology Division participates in three undergraduate training programs: (i) Great Lakes Colleges Association/Associated Colleges of the Midwest (GLCA/ACM Science Semester, (ii) Southern Colleges University Union Science Semester (SCUU), and (iii) Oak Ridge Associated Universities Summer Student Trainee (ORAU). Under the auspices of these organizations and in cooperation with Oak Ridge National Laboratory, outstanding college juniors are offered opportunities for independent research in the life sciences. Ten to twenty students who possess the educational qualifications and the potential for a successful scientific career spend 16 weeks (GLCA/ACM and SCUU) or 10 weeks (ORAU) doing research under the guidance of Biology Division staff members.

Although the principal purpose of the programs is to provide a training experience for the students, it often allows division staff members an opportunity to broaden their areas of research. Upon completion of their research activities in the laboratory, students prepare a formal scientific paper and present a talk on their work. The programs, in which about 500 students have participated during the past 20 years, have received the enthusiastic endorsement of the students and the members of the Biology Division.

Appendices

A. Advisory Committee — 1980

Dr. Bernard L. Horecker	Roche Institute of Molecular Biology
Dr. Boris Magasanik	Department of Biology, Massachusetts Institute of Technology
Dr. Dean R. Parker	DOE, Division of Biomedical and Environmental Research, Retired
Dr. Henry C. Pitot	McArdle Laboratory for Cancer Research, University of Wisconsin
Dr. Thomas Roderick	The Jackson Laboratory
Dr. Robert T. Schimke	Department of Biological Sciences, Stanford University
Dr. Bruce O. Stuart (ad hoc member, 1980)	Stauffer Chemical Company
Dr. Arthur C. Upton	New York University Medical Center, Institute of Environmental Medicine
Dr. Gerald N. Wogan	Department of Nutrition and Food Sciences, Massachusetts Institute of Technology

B. Seminar Programs

INTERNAL SEMINARS AND JOURNAL CLUBS

Aging Research Journal Club	Semimonthly
Biochemistry Journal Club	Semiweekly
Cancer Research Seminar	Weekly
Genetics Seminar	Weekly
Histopathology Slide Seminar	Biweekly
Mutagenesis Journal Club	Biweekly

SEMINARS BY OUTSIDE SPEAKERS

The following seminars were given in the Biology Division by scientists from research organizations in the United States and abroad during the period October 1, 1978, through May 31, 1980.

Speaker	Affiliation	Subject
Stuart A. Aaronson[a]	National Cancer Institute	Recent advances in the biology of mammalian RNA tumor viruses
M. M. Abdel-Monem	University of Minnesota	Modulation of polyamine biosynthesis
James Allen[b]	National Institute of Environmental Health Sciences	In vivo BrdU-dye studies of replication kinetics, sister-chromatid exchange, and meiotic exchange
William A. Arnold[c]	Oak Ridge National Laboratory	The first step in photosynthesis
William Au	M. D. Anderson Hospital and Tumor Institute	Cytogenetic assays for genetic toxicity
William Baird	The Wistar Institute	Metabolic activation of polycyclic aromatic hydrocarbons by rodent embryo cell cultures
Walter Balcavage	Indiana State Medical School	Role of aged pigment in cellular aging
David Baltimore[c]	Massachusetts Institute of Technology	Transforming mouse leukemia viruses
Eliezer Benjamini[a]	University of California, Davis	Antigens, cell surface receptors, and their interactions
Berit Helene Berg[d]	University of Bergen, Norway	Regulation of aminoacyl-tRNA synthetase activities in mouse uterus and liver following 17β-estradiol treatment
Richard Berlin	University of Connecticut Health Center	Endocytosis in cultured cells
P. M. Bhargava	Centre for Cellular and Molecular Biology, India	Control of cell division and malignant transformation by uptake of essential nutrients
Gopal M. Bhatnagar	Johns Hopkins Medical School	Epidermal contractile proteins
Rajendra S. Bhatnagar	University of California, San Francisco	Collagen: a primary molecular site of environmental toxicity

P. K. Bhattacharya	Indian Institute of Science, Bangalore	Design of affinity systems for biomedical problems
Gunter Blobel[c]	Rockefeller University	Intracellular pathways of newly synthesized protein
Peter M. Blumberg	Harvard Medical School	A pharmacological approach to the mechanism of action of phorbol ester tumor promoters
Lawrence Boone[a]	Roche Institute of Molecular Biology	Recombinant DNA clones of avian leucosis virus and analysis of proviral DNA synthesis
Carmia Borek[a]	Columbia University	Radiation carcinogenesis *in vitro*
James Bridges[a]	University of Surrey, England	Use of isolated hepatocytes in drug metabolism and toxicology
John Bywater	Basel Institute for Immunology, Switzerland	Immunological detection of a protozoan causing widespread disease among laboratory animals
F. W. Carlborg[a]	St. Charles, Illinois	Risk assessment for human carcinogens
Donald Cline	Burroughs Wellcome Laboratory	Comparative chemical mutagenesis. Can we make risk estimates?
Richard Courtney[a]	University of Tennessee, Knoxville	Biochemical and immunological studies of Herpes simplex virus proteins
Michel Daune[a]	Institut de Biologie Moleculaire et Cellulaire du CNRS, France	Structural modifications and protein recognition of DNA modified by N-2-acetylaminofluorene and its 7-iodo derivative
William C. Dewey	Colorado State University	Hyperthermia: A modality for cancer therapy and a tool for studying molecular and cellular biology
Michael S. Dickens[a]	Institute for Cancer Research, Philadelphia	Prevention by retinoid of chemical transformation of mouse mammary glands in whole organ culture
John DiGiovanni	The Wistar Institute	Polycyclic hydrocarbon metabolism in epidermal cells
Darrell Doyle	Roswell Park Memorial Institute	Turnover and biogenesis of the plasma membrane of mammalian cells
John W. Drake[e]	National Institute of Environmental Health Sciences	The theory of mutagenenesis

Russ DuFrain	Oak Ridge Associated Universities	Chemically induced cytogenetic damage in rabbit somatic cells and oocytes
Bernard Dujon[e]	Harvard University	Introns of the yeast mitochondrial genome and their functions
Udo H. Ehling	Gesellschaft fur Strahlen- und Umweltforschung MBH, Germany	Estimation of genetic risk with recessive and dominant mutations in mice
Gunther Eichhorn	National Institutes of Health	Chemistry of metal ion interactions with nucleic acids
Rosalie K. Elespuru	Frederick Cancer Research Center	Colorimetric phage induction assay for carcinogenic and cancer therapeutic agents
M. M. Elkind[a]	Argonne National Laboratory	Neoplastic transformation: Is it subject to repair?
Michael J. Evans[a]	SRI International	Cell renewal in the lung
Philip Farabaugh	Cornell University	DNA sequence analysis of the integration and excision of a transposable genetic element in yeast
James G. Farrelly[a]	Frederick Cancer Research Center	The metabolism of nitrosopyrrolidine and its relation to mutagenesis
Gerald R. Fink[e]	Cornell University	Transposition elements in yeast
L. M. Franks	Imperial Cancer Research Fund Laboratory, England	Aging and carcinogenesis <u>in vitro</u> – do we ask the right questions?
A. Freeman[a]	La Jolla Cancer Research Foundation	The growth and characterization of organotypic epithelial cells in culture
Elaine Fuchs	Massachusetts Institute of Technology	Keratin gene expression in differentiating epidermal cells
Gerhard Furstenberger	German Cancer Research Center, Heidelberg	Mechanism of action of phorbol ester tumor promoters
Arthur C. Giese	Stanford University	The photobiology of the pigmented ciliate Blepharisma

T. Glover	University of Hawaii	Diphtheria — toxin resistance in human cells and heritable X-chromosome-fragile sites
J. Gluzman[a]	Cold Spring Harbor Laboratory	The use of mutants for the analysis of SV40 replication and cell transformation
Joel S. Greenberger[a]	Harvard Medical School	Effects of murine retroviruses on long-term *in vitro* hemopoiesis
Philip Grover[a]	Chester Beatty Research Institute, England	The metabolic activation of methyl-substituted carcinogenic polycyclic hydrocarbons
Barbara Hamkalo	University of California, Irvine	Structure of mammalian chromosomes
David Haney	Northwestern University	Chemical studies on hemoglobin: (1) Glycosylation in HbA1C, (2) Molecular aggregation in HbS
R. E. Harrington	University of Nevada	New insights into the DNA packaging problem
Bruce S. Hass	Argonne National Laboratory	Genetic studies with continuous cultures
Ira Herskowitz[e]	University of Oregon	Gene control by "conventional" and "unconventional" means in yeast: static and mobile genes
Leroy E. Hood[c]	California Institute of Technology	(1) Antibody diversity and DNA rearrangement (2) Structure and evolution of gene products of the major histocompatibility complex
Bernard L. Horecker	Roche Institute of Molecular Biology	Biosynthesis of thymic and opioid peptides
James C. Hunt	University of Tennessee, Memphis	Future directions of the University of Tennessee College of Medicine
Tadashi Inoue[b]	National Institute of Genetics, Japan	DNA repair enzymes for gamma-ray-induced damages in bacterial and human cells
Olav Hilmar Iversen	University of Wisconsin	Epidermal carcinogenesis. Some heretic thoughts on the two-stage theory
John Kao[a]	National Institute of Environmental Health Sciences	*In vivo* and *in vitro* studies on the teratogenicity of anticonvulsants
Marvin A. Kastenbaum	Tobacco Institute	Credible conjecture or scientific truth

Shirley L. Kauffman[a]	State University of New York, Brooklyn	Bronchiolo-alveolar tumors in mice
Celik Kayalar	Massachusetts Institute of Technology	Bioenergetic aspects of protein function in membranes
G. Kellermann	University of Wisconsin	Relationship between in vitro and in vivo human drug metabolism
Francoise Kelly	Pasteur Institut, France	The effect of SV40 virus transformation on early mouse embryo development
H. Kerstin[d]	Erlangen, Germany	Specific modifications in tRNA: influence on tRNA structure and function
Robert S. Lake	Children's Hospital Medical Center, Akron	Chemical carcinogenesis studies in organotypic human cell cultures
Jerry Last	University of California, Davis	In vitro approaches to the study of mucus glycoprotein structure
James F. Leary	University of Rochester Medical Center	Use of cytofluorograph for screening and separating white blood cells
Wallace Le Sturgeon[f]	Vanderbilt University	HnRNA packaging: composition and structure of nuclear 40 S particles
Albert P. Li	Lovelace Foundation	Effects of diesel exhaust extracts on cytotoxicity and mutagenicity
Mike W. Lieberman[a]	Washington University School of Medicine	Role of chromatin structure in DNA repair
William Lijinsky	Frederick Cancer Research Center	Nitrosamine mutagenesis and carcinogenesis
Bjorn Lydersen[a]	University of Colorado Medical Center	Nonhistone chromosomal proteins of normal and transformed cells
Peter A. Mahler[a]	University of Wisconsin	Rat mammary epithelial cell survival following fast neutron irradiation
Clement Markert[e]	Yale University	Analysis of cell differentiation by techniques of genetic and embryological engineering
James Mascarello[b]	Children's Hospital and Health Center, San Diego	A Chinese hamster cell line with defective succinate dehydrogenase: the assignment of a gene for S.D.H. to human chromosome 1

Robert M. McAllister[a]	Children's Hospital of Los Angeles	Transfection by endogenous and exogenous baboon type-C viral DNAs
D. B. McGregor	Inveresk Research International, Scotland	Mutagenicity testing of gases and vapours in bacteria
Charles G. Miller[f]	Case Western Reserve University	Genetic analysis of intracellular proteolysis in bacteria
Elizabeth C. Miller[c]	University of Wisconsin Medical Center	Some aspects of the metabolic activation of chemical carcinogens
Oscar L. Miller, Jr.[f]	University of Virginia	Ultrastructural aspects of genetic activity
James B. Mitchell[b]	National Cancer Institute	Dose-rate effects on mammalian cells exposed to ionizing radiation
Theodore W. Munns[a]	St. Louis University	Antibody — nucleic acid complexes. Immunochemical approaches for the isolation and characterization of nucleic acids
Uma Nandi	University of Pennsylvania	Interaction of metal ions with nucleic acids: application to chemotherapy
A. T. Natarajan	University of Leiden, The Netherlands	DNA lesions responsible for X-ray-induced chromosome aberrations and UV-induced sister chromatid exchanges
Nancy L. Oleinick[g]	Case Western Reserve University	Repair of total and ribosomal DNA in gamma-irradiated Tetrahymena: relation to radiation-modified protein and RNA synthesis
Leslie E. Orgel[c]	The Salk Institute	(1) Prebiotic chemistry and the origins of life (2) Template-directed polynucleotide synthesis
Henry C. Outzen[a]	The Jackson Laboratory	Immunomodulation of oncogenesis
Inder J. Paika[b]	The Children's Hospital, Boston	Analysis of *in vivo*-induced sister chromatid exchanges
W. W. Parson[f]	University of Washington	Early steps in bacterial photosynthesis
Meyrich Peak	Argonne National Laboratory	Formation of single-strand breaks in transforming DNA by near-UV radiation

R. A. Pelroy	Battelle Pacific Northwest Laboratories	Mutagenicity evaluation of synthetic fuel
Michael J. Plewa[b]	University of Illinois	The use of plant systems in environmental mutagenesis
Theodore Pincus	The Wistar Institute	Studies on endogenous murine leukemia viruses
Henry Pitot	University of Wisconsin	Two-stage liver carcinogenesis
A. P. Polednak[a]	Oak Ridge Associated Universities	Some epidemiological studies of radiation-exposed workers
Morris Pollard[a]	University of Notre Dame	Animal model systems for cancer research
Ian Pragnell	Beatson Institute for Cancer Research, Scotland	Myeloproliferative sarcoma virus: a murine sarcoma virus which also interacts with hemopoietic cells
Ulf Rannug	University of Stockholm, Sweden	The use of different metabolizing systems in elucidating the mutagenic effects of 1,2-dichloroethane on Salmonella
John J. Reiners, Jr.[b]	Baylor College of Medicine	Transcriptional and posttranscriptional modulation of cytoplasmic RNAs in regenerating liver and Novikoff hepatoma
Robert D. Reynolds	U.S. Department of Agriculture	Studies on the regulation of tyrosine aminotransferase
Mark Robbins[a]	University of Minnesota	Effect of interferon-inducing agents on the developmental hepatic drug metabolism
Robert G. Roeder	Washington University	Transcription of eukaryotic genes in reconstituted systems
Jean Rommelare[a]	Yale University	Parvoviruses as probe of replicative bypass mechanisms in UV-irradiated mammalian cells
Fritz M. Rottman[d]	Michigan State University	Methylation analysis of mRNA and partial characterization of bovine prolactin mRNA sequence
Brahma P. Sani[a]	Southern Research Institute, Birmingham	Role of retinoic acid-binding protein in differentiation and control of tumorigenesis

Robert Schimke[c]	Stanford University	Gene amplification and metotrexate resistance in cultured mammalian cells
John Scribner	Pacific Northwest Research Foundation	Studies on the mechanism of action of aromatic amine carcinogens
Claus H. Schröder	Deutsches Krebsforschungszentrum Institut für Virusforschung, GFR	Homologous interference in serial high multiplicity passages of Herpes simplex virus
J. D. Seidman[e]	National Institute of Child Health and Human Development	The arrangement of the mouse immunoglobulin light chain genes
Michel Seman	Research Institute of Molecular Biology, France	Role of H-2 genes in the regulation of class-specific immunoglobulin production induced by sheep red blood cells
Jane K. Setlow[a]	Brookhaven National Laboratory	ATP-dependent nuclease from Haemophilus
Charles J. Sherr	National Cancer Institute	Molecular cloning of DNA intermediates of Snyder-Theilen feline leukemia-sarcoma viruses
S. J. Singer[c]	University of California at San Diego	(1) Evidence for a large internal pressure in biological membranes (2) Molecular interactions across membranes
Hamilton O. Smith[f]	Johns Hopkins University	A specific sequence in Haemophilus DNA governs uptake during transformation
John Smythies[a]	University of Alabama at Birmingham	Some molecular bases for tumor promotion
Dieter Söll	Yale University	The arrangement and transcription of eukaryotic tRNA genes
J. Soll[d]	Hannover, Germany	Biosynthesis of prenylquinones
Heinz Walter Thielmann[d]	Deutsches Krebsforschungszentrum Institut für Biochemie, GFR	Carcinogen-induced DNA repair in nucleotide-permeable Escherichia coli cells and in fibroblasts
Raymond Tice	Brookhaven National Laboratory	Cytogenetic effects of benzene and diethylstilbestrol in murine bone marrow

J. R. Totter[a]	Oak Ridge Associated Universities	Epidemiological approaches to determining spontaneous cancer rates
Branko Vitale	Institute "R. Bošković", Yugoslavia	Studies of the mechanisms of allogeneic disease in mice
R. C. von Borstel[b]	The University of Alberta, Canada	Spontaneous mutations from DNA repair
Peter H. von Hippel[c]	University of Oregon	Specific and nonspecific interactions of genome regulatory proteins with DNA
Charles A. Waldrer	University of Colorado Medical Center	Immunogenetics of human cell surface antigens and its application to screening chromosomal nondisjunction by physical and chemical agents
Richard Weisenberg	Temple University	Mechanisms of microtubule assembly and regulation
Herbert Weissbach[f]	Roche Institute of Molecular Biology	Attempts to express a bacterial gene in a defined system
Paul Wigler[d]	University of Tennessee Center for Health Sciences, Knoxville	Trans-efflux inhibition of nucleoside transport by mercury in yeast
J. E. Womack	Texas A & M University	Neuraminidase deficiency and processing genes in the mouse
Fritz Würgler	University of Zurich, Switzerland	Mutation induction in repair-deficient strains of Drosophila
C. C. Yang	National Tsing Hua University, Taiwan	Structure-function and immunochemical studies on cobrotoxin

[a]Cancer Research Seminar
[b]Genetics Seminar
[c]Distinguished Lecturer Series
[d]Biochemistry Journal Club
[e]Genetics and Developmental Biology Seminar Series
[f]Molecular and Cellular Sciences Program Seminar Series
[g]Program in Radiation Biology and University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences

C. Annual Research Conferences

The thirty-second annual research conference of the Biology Division was held in Gatlinburg, Tennessee, April 15-19, 1979. The subject was The Scientific Basis of Toxicity Assessment, and Hanspeter R. Witschi was chairman of the organizing committee. The proceedings of the meeting were published in 1980 by Elsevier/North-Holland Biomedical Press as Volume 6 in their Developments in Toxicology and Environmental series.

The 1980 symposium on DNA-Multiprotein Interactions in Transcription, Replication, and Repair was held in Gatlinburg March 24-27, 1980. Robert K. Fujimura was chairman of the organizing committee, and the proceedings will be published in the near future by Academic Press in their Progress in Nucleic Acid Research and Molecular Biology Series.

The 1981 meeting on Molecular and Cellular Mechanisms of Mutagenesis is scheduled to be held in Gatlinburg April 6-9. The symposium will focus on several diverse DNA-related phenomena that contribute to or affect the production of mutations in various organisms. The talks will emphasize fundamental molecular mechanisms as regulated by cellular (genetically controlled) functions. J. F. Lemontt is chairman of the organizing committee, with W. M. Generoso as cochairman. Additional information about the meeting can be obtained by writing directly to Dr. Lemontt, Biology Division, P. O. Box Y, Oak Ridge, TN 37830.

D. Extramural Activities

1. Officer of Society

D. Billen	– Councilor, Radiation Research Society
J. S. Cook	– President, Society of General Physiologists, 1979-1980
J. L. Epler	– Councilor, Environmental Mutagen Society, 1979-present
R. J. M. Fry	– Councilor, Radiation Research Society, 1975-1978 Councilor, International Association for Radiation Research, 1974-1979
R. F. Kimball	– Councilor, Environmental Mutagen Society
S. P. Leibo	– Vice President, Society for Cryobiology, 1979
J. W. Longworth	– President, American Society for Photobiology, 1978 Past President, American Society for Photobiology, 1979 Executive Committee, American Society for Photobiology, 1980 Executive Board, International Association for Photobiology, 1976-1980
L. B. Russell	– Councilor, Environmental Mutagen Society, 1977-1980 Executive Committee, Environmental Mutagen Society, 1979-1980
G. Sega	– Councilor, Environmental Mutagen Society, 1978-1981

2. Society Committees

N. K. Clapp — Education and Training Committee, Radiation Research Society

J. S. Cook — USA National Committee for the International Union of Physiological Sciences (Vice-Chairman), 1977-1982

F. C. Hartman — Nominating Committee, American Society of Biological Chemists, 1980

F. T. Kenney — Advisory Committee on Personnel for Research, American Cancer Society, 1971-present

J. W. Longworth — American Society for Photobiology Subcommittee for 1984. IX International Congress on Photobiology (Chairman)
International Scientific Program Committee for VIII International Congress on Photobiology, 1978-1980
Program Committee (Chairman), Joint National Meeting of Biophysical Society and American Physical Society, 1978

R. A. Popp — Laboratory Animal Data Bank Evaluation Committee, Federation of American Societies for Experimental Biology and Medicine

L. B. Russell — Nominating Committee (Chairman), Environmental Mutagen Society, 1979-1980
Nominating Committee (Chairman), Genetics Society of America, 1979-1980

D. M. Skinner — Nominating Committee, American Association for the Advancement of Science (Co-chairman, 1979), 1977-1979
Representative to AAAS, Society of General Physiology, 1980-1983
Nominating Committee, Biophysical Society, 1980
Committee on Equal Opportunities for Women, American Society for Biological Chemistry, 1980-1983

3. Advisory Committees

D. Billen — Cancer Research Manpower Review Committee, National Cancer Institute

J. L. Epler — Review Panel on Diesel Emissions Health Effects, Environmental Protection Agency, 1978
Interagency Program on US/USSR Oil Shale Research Activities

R. J. M. Fry — U.S. National Committee for Photobiology, National Academy of Sciences-National Research Council, 1978-present

F. C. Hartman — Study Panel, U.S. Department of Agriculture, 1979

C. A. Heckman — Advisory Committee on Cell Biology, National Science Foundation, 1977-present

A. W. Hsie — Scientific Adviser for the Center for Biological Research, Academia Sinica, Republic of China, 1977-1979
Member of Scientific Directorate, Coordinating Council for Cancer Research, Villejuif, France, 1978-present
Committee on Evaluation of Chinese Hamster Ovary Cell Mutational System (Chairman), Gene-Tox Task, Environmental Protection Agency, 1978-present
Committee on Evaluation of Body-Fluid Analysis, Gene-Tox Task, Environmental Protection Agency, 1979-present

Committee on Host-Mediated Assay, Gene-Tox Task, Environmental Protection Agency, 1979-present
Committee on Federal Research into the Biological Effects of Ionizing Radiation, National Institutes of Health, 1975
Special Study Section, Environmental Health Sciences, National Institutes of Health, 1980

E. Huberman — Cause and Prevention Scientific Review Committee, National Institutes of Health, 1978-1981
Committee on Chemical Environmental Mutagens, National Academy of Sciences, 1979-1981
Committee on Specific Gene Mutations in V79 Cells (Chairman), Gene-Tox Task, Environmental Protection Agency, 1979-1980
Toxic Substances Subcommittee of the Executive Committee of the Science Advisory Board, Environmental Protection Agency, 1980-present

F. T. Kenney — International Advisory Board, Cancer Biochemistry Biophysics, 1971-present

R. F. Kimball — Advisory Council, National Institute of Environmental Health Sciences

J. W. Longworth — U.S. National Committee for Photobiology (Chairman), National Academy of Sciences-National Research Council, 1976-1978

P. Mazur — Advisory Committee on Blood Program Research, American National Red Cross, 1976-1979
Germplasm Resources Committee, National Academy of Sciences, 1976-1978
Subcommittee on Biology and Medicine, Polar Programs, National Science Foundation, 1978-present

R. J. Preston — Committee on In Vivo/In Vitro Cytogenetics, Gene-Tox Task (Chairman), Environmental Protection Agency, 1979-present

L. B. Russell — Committee on the Biological Effects of Ionizing Radiation (Genetic Effects, Somatic Effects), National Academy of Sciences
Committee I, International Commission for Protection Against Environmental Mutagens and Carcinogens, 1977-present
Committees (2) on Gene-Tox Task (Chairman), Environmental Protection Agency, 1979-present
Committee on Mutagenesis Tests with Whole Mammals (Chairman), International Agency for Research on Cancer, 1979

G. Sega — Committee on DNA Repair, Gene-Tox Task, Environmental Protection Agency, 1979-present

D. M. Skinner — Member of the Corporation, Marine Biological Laboratory, Woods Hole, 1971-present

J. B. Storer — National Council on Radiation Protection and Measurement: Council member, Board of Directors, Scientific Committee on Basic Radiation Criteria, Scientific Committee on Biological Aspects of Basic Radiation Criteria
Advisory Committee on Radiation Effects Research Foundation, National Academy of Sciences
U.S. Delegate to United Nations Scientific Committee on the Effects of Atomic Radiation, U.S. State Department
Scientific Council, Radiation Effects Research Foundation

H. P. Witschi — Toxicology Study Section, National Institutes of Health, 1980-1984
Scientific Review Panel for Health Research, Office of Research and Development, Environmental Protection Agency
Diesel Impact Study Committee, Panel on Health Effects, National Research Council
Board of Directors, Toxicology Laboratory Accreditation Board, Inc.

4. Editorial Boards

H. I. Adler — Advances in Radiation Biology, Academic Press, 1974-1980
Radiation Research, 1980-1983

D. Billen — Radiation Research (Editor-in-Chief), 1979-present

J. L. Epler — Mutation Research, 1978-present

R. J. M. Fry — Cell and Tissue Kinetics, 1964-1979

F. C. Hartman — BioScience, 1980-1982

A. W. Hsie — Mutation Research, 1976-present

E. Huberman — Journal of Teratogenesis, Carcinogenesis and Mutagenesis, 1980-present

J. F. Lemontt — Mutation Research, 1979-present

P. Mazur — Revue Francaise de Transfusion et Immunohématologie, 1979-present
Cryo-Letters, 1979-present
Cryobiology

R. A. Popp — Journal of Experimental Zoology, 1975-1978
Differentiation, 1978-1980

R. J. Preston — Mutation Research Letters, 1980-present
Mutation Research, 1976-present
Teratogenesis, Carcinogenesis and Mutagenesis. 1979-present
Environmental and Experimental Botany, 1979-present
Environmental Mutagenesis, 1979-present

L. B. Russell — Mutation Research, 1976-present
Environmental Mutagenesis, 1978-present

D. M. Skinner — Growth, 1979-1982

H. P. Witschi — Toxicology and Applied Pharmacology
Toxicology

5. Awards

R. F. Grell	– Elected to Hunter College Hall of Fame, 1978
F. C. Hartman	– Pfizer Award in Enzyme Chemistry, 1979
A. W. Hsie	– Distinguished Alumni Service Award, Indiana University, 1980
D. E. Olins	– Alexander von Humboldt Senior U.S. Scientist Award, 1979
J. D. Regan	– Senior Fellow of the Japan Society for the Promotion of Science, 1980 Medal of the National Cancer Institute of Japan, 1980 Medal of Tokai University, 1980
L. B. Russell	– Elected to Hunter College Hall of Fame, 1979

E. Abstracts for Technical Meetings

ABSTRACTS FOR TECHNICAL MEETINGS HELD
OCTOBER 1, 1978 – MAY 31, 1980

Adler, Howard I., Alice A. Hardigree, and T. K. Rao. An attempt to develop a rapid and miniaturized microbial assay for mutagens. Workshop on Status of DOE Program Involving Development and Use of Short-Term Bioassays for Identifying Toxic Agents in Complex Mixtures and Prospects for Standardization, Boca Raton, Florida, November 11-15, 1978.

Amsler, Kurt. Analysis of a variant clone of BHK21 cells with decreased rate of 2-deoxy-glucose transport. Society for Experimental Biology and Medicine, Southeastern Section, Oak Ridge, Tennessee, October 24-27, 1979.

Amsler, Kurt and John S. Cook. Isolation and partial characterization of a variant clone of BHK21 cells with decreased capability to transport 2-deoxyglucose. American Society for Cell Biology, Toronto, Canada, November 4-8, 1979.

Basu, S. P. and J. R. Einstein. Crystal and molecular structure of bulbocapnine methiodide. American Crystallographic Association, Honolulu, Hawaii, March 27-30, 1979.

Becker, John H. and John S. Cook. The effect of the tumor-promoting agent TPA on amino acid transport in induced and uninduced murine erythroleukemia cells. American Society for Cell Biology, San Antonio, Texas, November 4-8, 1978.

Bernstine, Edward G. Genetic regulation of mitochondrial malic enzyme in mouse brain. Workshop on Biochemical Genetics. Jackson, Wyoming, February 7-10, 1979.

Berry, D. L. and T. J. Slaga. Metabolic conversion of 12-O-tetradecanoylphorbol-13-acetate (TPA) by mouse liver microsomes, adult, and newborn mouse skin and the uptake and metabolism in newborn and adult epidermal cells in culture. XII International Cancer Congress, Buenos Aires, Argentina, October 5-11, 1978.

Birchler, James. Alterations of enzyme levels in segmental aneuploids of Drosophila and maize. Joint Meeting, Genetics Society of Canada/Genetics Society of America, Edmonton, Alberta, Canada, August 20-22, 1979.

Birchler, James A. Genetic studies on trans-acting regulatory effects in higher eukaryotes as revealed by chromosomal imbalance. Twelfth Miami Winter Symposium, Miami, Florida, January 7-11, 1980.

Blevins, R. Dean and J. D. Regan. Nitrosated methylcarbamate insecticides: Effect on the DNA of human cells and on mutants of Salmonella typhimurium LT2. XIth International Congress of Biochemistry, Toronto, Canada, July 8-13, 1979.

Brewen, Bobbie and C. E. Nix. Mutagenicity of aliphatic nitrosamines in Drosophila melanogaster. Environmental Mutagen Society, New Orleans, Louisiana, March 8-12, 1979.

Brown, P. C. and John Papaconstantinou. Modulation of albumin synthesis and corresponding mRNA levels in cultured mouse hepatoma cells by hydrocortisone and cAMP. American Society for Cell Biology. San Antonio, Texas, November 4-8, 1978.

Browne, Carole L. and James N. Dumont. Hormonal regulation of intercellular communication between the oocyte and follicle cells of Xenopus laevis. American Society for Cell Biology, San Antonio, Texas, November 4-8, 1978.

Broyde, S. and B. Hingerty. Pyrimidine (3'-5') purine base sequence preference for intercalation into RNAs. Biophysical Society, Atlanta, Georgia, February 25-28, 1979.

Broyde, S. and B. Hingerty. The coil form of poly rU: A model composed of low energy conformers that matches experimental properties. Conference on Stereo Dynamics of Molecular Systems, Albany, New York, April 23-24, 1979.

Brummett, Anna Ruth and James N. Dumont. Sperm penetration and cortical reaction in Fundulus. American Society for Cell Biology, Toronto, Canada, November 4-8 1979.

Bryan, Philip N. Core nucleosomes by digestion of reconstructed histone-DNA complexes. Biophysical Society, Atlanta, Georgia, February 25-28, 1979.

Bunick, G. J., T. J. Laughlin, J. K. W. Mardian, D. E. Olins, and H. E. Thiessen. X-ray diffraction, electron microscopy, and optical diffraction studies of nucleosome crystals. Symposium on DNA-Multiprotein Interactions in Transcription, Replication, and Repair, Gatlinburg, Tennessee, March 24-27, 1980.

Burd, P. R., S. Mitra, and R. C. Bates. Restriction enzyme map of the bovine parvovirus genome. American Society for Microbiology, Miami Beach, Florida, May 11-16, 1980.

Butler, A. P. and D. E. Olins. Fluorescence studies of inner histones. Biophysical Society, Atlanta, Georgia, February 25-28, 1979.

Bynum, John W. and Elliot Volkin. Poly(U) sepharose analysis of chromatin-associated RNA. Federation of American Societies for Experimental Biology (American Society of Biological Chemists), Dallas, Texas, April 1-10, 1979.

Bynum, John W. and Elliot Volkin. Pulse-chase analysis of different poly(A) RNA species isolated from chromatin-associated RNA. XIth International Congress of Biochemistry, Toronto, Canada, July 8-13, 1979.

Cacheiro, L. H., E. H. Perkins, P. L. Glover, and P. Mazur. Preliminary attempts to assess the ability of fetal mouse thymuses to function after freezing and thawing. Society for Cryobiology, Atlanta, Georgia, September 30-October 4, 1979.

Cacheiro, Nestor L. A. The Oak Ridge series of male sterile anomalies. Workshop on chromosomal Aspects of Male Sterility in Mammals, Harwell, Oxford, England, October 24-26, 1979.

Cacheiro, Nestor L. A. and Ethel L. Russell. Causes of sterility in male mice derived from germ cells treated in spermatogonial or postspermatogonial stages with cyclophosphamide. Environmental Mutagen Society, Nashville, Tennessee, March 17-20, 1980.

Carrier, W. L., D. P. Smith, and J. D. Regan. The effect of arabinofuranosyl cytosine (ara-C) on pyrimidine dimer incision and excision in the DNA of UV-irradiated normal skin fibroblasts. Biophysical Society, Atlanta, Georgia, February 25-28, 1979.

Carrier, W. L., D. P. Smith, and J. D. Regan. Ultraviolet light dose response of pyrimidine dimer excision in human cells. 7th Annual Meeting of the American Society for Photobiology, Carmel, California, June 24-28, 1979.

Carrier, William L., David P. Smith, and James D. Regan. The repair of ultraviolet light damage in the DNA of human cells. Society for Experimental Biology and Medicine, Southeastern Section, Nashville, Tennessee, November 2-3, 1978.

Chang, Susan S. and R. O. Rahn. A nonradioactive assay for thymidine dimers in UV-irradiated DNA. Biophysical Society, Atlanta, Georgia, February 25-28, 1979.

Christie, N. T. and D. M. Skinner. Nonrandom sequence alterations in a highly repetitive fraction of DNA from the red crab, Geryon quinquedens. American Society for Cell Biology, Toronto, Canada, November 4-8, 1979.

Christie, Nelwyn T. and Dorothy M. Skinner. The primary sequence of DNA of satellite frequency from the red crab, Geryon quinquedens. American Society for Cell Biology, San Antonio, Texas, November 4-8, 1978.

Clapp, Neal K., David S. Barnes, David A. Scott, Donna L. Oberst, and Susan G. Berry. Effect of dietary bran on 1,2-dimethylhydrazine (DMN) tumorigenesis in rats. American Association for Cancer Research, San Diego, California, May 28-31, 1980.

Clapp, Neal K., Lou C. Satterfield, and Norman D. Bowles. A marked increase in survival in both irradiated and nonirradiated mice after butylated hydroxytoluene (BHT). 6th International Congress of Radiation Research, Tokyo, Japan, May 13-19, 1979.

Clark, Jeffrey N. and Ann C. Marchok. Cellular differentiation and mucous glycoprotein synthesis by cloned tracheal epithelial tumor cells exposed to retinyl acetate in vitro. American Society for Cell Biology, Toronto, Canada, November 4-8, 1979.

Constantin, Milton J., Karen Lowe, T. K. Rao, Frank W. Larimer, and James L. Epler. The detection of potential genetic hazards in complex environmental mixtures using plant cytogenetics and microbial mutagenesis assays. Symposium on Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures, Williamsburg, Virginia, March 4-7, 1980.

Cumming, R. B., M. L. Bauer, R. K. Genung, D. D. Willis, N. E. Lee, W. D. Bostick, G. B. Johnson, and J. E. Mrochek. The development of a fast rotary electrophoresis system with capacity for high sample throughput and great resolving power. Symposium on Advanced Analytical Concepts for the Clinical Laboratory, Oak Ridge, Tennessee, April 26-27, 1979.

Cumming, R. B. and Teresa A. Michaud. Mutagenic effects of inhaled ethylene oxide in male mice. Environmental Mutagen Society, New Orleans, Louisiana, March 8-12, 1979.

Dalbey, W. E., P. Nettesheim, R. Griesemer, J. E. Caton, and M. R. Guerin. Chronic inhalation of cigarette tobacco smoke by Fischer rats. Society of Toxicology, Washington, D.C., March 9-13, 1980.

Davidson, K. A., J. M. Holland, J. W. Hall, and L. C. Gipson. Endocrine morphology and reproductive function in athymic nude mice. 3rd International Workshop on Nude Mice, Bozeman, Montana, September 6-9, 1979.

Dodson, Lori and Charles T. Hadden. Post-replication repair in UV-irradiated Bacillus subtilis. 23rd Wind River Genetics Conference, Estes Park, Colorado, June 12-15, 1979.

Dodson, Lori A. and Warren E. Masker. Development of an in vitro system for the detection of mutagenesis. Kentucky-Tennessee Branch, American Society for Microbiology, Memphis, Tennessee, October 4-6, 1979.

Dorsett, Dale L., John J. Yim, and K. Bruce Jacobson. Biosynthesis of drosopterins in Drosophila melanogaster. Federation of American Societies for Experimental Biology (American Society of Biological Chemists), Dallas, Texas April 1-10, 1979.

Einstein, J. R., S. P. Basu, B. E. Hingerty, and C. H. Wei. Structure of a high sulfur-containing soybean trypsin inhibitor. American Crystallographic Association, Boston, Massachusetts, August 19-24, 1979.

Epler, J. L., T. K. Rao, C.-h. Ho, and M. R. Guerin. Identification of mutagenic components of basic fractions from crude oils. Environmental Mutagen Society, New Orleans, Louisiana, March 8-12, 1979.

Epler, J. L., T. K. Rao, F. W. Larimer, M. R. Guerin, C.-h. Ho, and B. R. Clark. Isolation and identification of mutagenic polycyclic aromatic amines in synthetic crude oils. Environmental Mutagen Society, Nashville, Tennessee, March 17-20, 1980.

Epler, J. L., W. Winton, A. A. Hardigr F. W. Larimer. The appropriate use of genetic toxicology in industry. Sympos the Scientific Basis of Toxicity Assessment, Gatlinburg, Tennessee, April 15-1 .

Filler, Ron and Sarah Garner-Shinpock. The metabolism of benzo[a]pyrene by murine embryonal carcinoma cells. American Association for Cancer Research, San Diego, California, May 28-31, 1980.

Filler, Ron and Sarah Shinpock. In vivo restriction of differentiational potential of mouse teratocarcinoma cells. 21st Southeastern Developmental Biology Conference, Fontana, North Carolina, April 5-7, 1979.

Fischer, S. M. and T. J. Slaga. In vitro human and murine epidermal cell transformation with chemical carcinogens. XII International Cancer Congress, Buenos Aires, Argentina, October 5-11, 1978.

Francis, Andrew A. and James D. Regan. The effect of hydroxyurea on DNA repair in UV-irradiated human cells. Biophysical Society, Atlanta, Georgia, February 25-28, 1979

Friedman, J. and E. Huberman. The involvement of post-replication repair in the induction of mutation by methylating agents in Chinese hamster cells. American Association for Cancer Research, San Diego, California, May 28-31, 1980.

Frim, J. and P. Mazur. Cryopreservation of human granulocytes using glycerol. Society for Cryobiology, Atlanta, Georgia, September 30-October 4, 1979.

Fry, R. J. M. Radiation carcinogenesis. Medical Education Course on the Risks and Benefits of Radiation Exposure, Chicago, Illinois, October 24, 1979.

Fry, R. J. M. and J. B. Storer. Radiation toxicology. Symposium on the Scientific Basis of Toxicity Assessment, Gatlinburg, Tennessee, April 15-19, 1979.

Fujimura, Robert K. Factors involved in phage T5 DNA replication in vitro: Resolution into two different fractions. XIth International Congress of Biochemistry, Toronto, Canada, July 8-13, 1979.

Fujimura, R. K., S. K. Das, D. P. Allison, and B. C. Roop. Replication of linear duplex DNA in vitro with bacteriophage T5 DNA polymerase. Symposium on DNA-Multiprotein Interactions in Transcription, Replication, and Repair, Gatlinburg, Tennessee, March 24-27, 1980.

Fuscoe, J. C., J. P. O'Neill, and A. W. Hsie. Induction of reverse mutations in Chinese hamster ovary (CHO) cells. Environmental Mutagen Society, Nashville, Tennessee, March 17-20, 1980.

Fuscoe, J. C., J. P. O'Neill, and A. W. Hsie. Mutation inactivation of ICR compounds complicates interrelationship of mutagenesis and carcinogenesis. American Association for Cancer Research, San Diego, California, May 28-31, 1980.

Fuscoe, James C., J. Patrick O'Neill, and Abraham W. Hsie. Structure-activity relationship of 19 antitumor ICR compounds in CHO/HGPRT system. Environmental Mutagen Society, New Orleans, Louisiana, March 8-12, 1979.

Generoso, W. M., C. W. Sheu, and R. M. Gryder. Studies on induction of dominant-lethal mutation and heritable translocation with ethylene oxide male mice. Environmental Mutagen Society, Nashville, Tennessee, March 17-20, 1980.

Gill, J. S. and H. I. Adler. Unstable division-promoting factors of filamentous Escherichia coli. Kentucky-Tennessee Branch, American Society for Microbiology, Memphis, Tennessee, October 4-6, 1979.

Glover, P. L., R. Chervenak, and E. H. Perkins. Lymphocyte proliferation in young adult and old mice. Gerontological Society, Washington, D.C., November 25-29, 1979.

Glover, P. L., R. Spector, R. Chervenak, and E. H. Perkins. Effect of cortisone on antigen and mitogen-induced proliferative responses of popliteal lymph node lymphocytes from young and old BALB/c mice. Federation of American Societies for Experimental Biology, Immunology, Anaheim, California, April 13-18, 1980.

Grell, R. F. The origin of meiotic nondisjunction. NIEHS Workshop on Systems to Detect Induction of Aneuploidy by Environmental Mutagens, Savannah, Georgia, November 5-8, 1978.

Grell, R. F. Meiotic recombination within heterochromatin of Drosophila melanogaster. Joint Meeting, Genetics Society of Canada/Genetics Society of America, Edmonton, Alberta, Canada, August 20-22, 1979.

Haas, B., M. Uziel, and J. K. Selkirk. Effects of benzo[a]pyrene on nucleic acids of hamster embryo fibroblasts during the cell cycle. Federation of American Societies for Experimental Biology, Physiology, Anaheim, California, April 13-18, 1980.

Hartman, Fred C. Design of affinity labels for enzymes of carbohydrate metabolism. Federation of American Societies for Experimental Biology (American Society of Biological Chemists), Dallas, Texas, April 1-10, 1979.

Hartman, Fred C. Affinity labels for enzymes of carbohydrate metabolism. 178th American Chemical Society Meeting, Washington, D.C., September 9-14, 1979.

Haschek, W. M., K. R. Meyer, R. L. Ullrich, and H. P. Witschi. Pulmonary fibrosis - A possible mechanism. Federation of American Societies for Experimental Biology (American Physiological Society), Dallas, Texas, April 1-10, 1979.

Haschek, Wanda and H. P. Witschi. On the pathogenesis of toxic lung fibrosis. Society of Toxicology, New Orleans, Louisiana, March 9-15, 1979.

Haschek, Wanda M. and Hanspeter Witschi. Pulmonary toxicity of coal liquefaction distillate product. 19th Annual Hanford Life Sciences Symposium, Richland, Washington, October 22-24, 1979.

Haschek, W. M., H. P. Witschi, and M. R. Boyd. Pulmonary and extrapulmonary toxicity of 3-methylfuran in mice. Society of Toxicology, Washington, D.C., March 9-13, 1979.

Haschek, W. M., H. P. Witschi, and M. R. Boyd. Pulmonary toxicity of 3-methylfuran. American College of Veterinary Pathology, Respiratory Disease Section, Denver, Colorado, December 4-8, 1979.

Heckman, C. A., A. D. Wei, and F. A. Pitlick. Turnover of the cell surface coat in human fibroblasts after subculture. American Society for Cell Biology, Toronto, Canada, November 4-8, 1979.

Hingerty, B. E. and S. Broyde. Multistate model for the random coil form of poly(rU). Biophysical Society, Atlanta, Georgia, February 25-28, 1979.

Hingerty, B. E., J. R. Einstein, and C. H. Wei. The crystal structure of adeninium nitrate hemihydrate. A model for poly(rAH^+). American Crystallographic Association, Eufaula, Alabama, March 17-21, 1980.

Hinkle, N. F., C. A. Chambers, and D. M. Skinner. The sequences of two unrelated cryptic satellites in the DNA of a hermit crab. American Society for Cell Biology, Toronto, Canada, November 4-8, 1979.

Hirsch, G. P., J. M. Holland, and R. A. Popp. Autoradiographic analysis of unscheduled DNA synthesis in vivo in the mouse seminal vesicle. 31st Annual Meeting of Gerontological Society, Dallas, Texas, November 16-20, 1978.

Ho, T., A. A. Hardigree, F. W. Larimer, C. E. Nix, T. K. Rao, S. C. Tipton, and J. L. Epler. Comparative mutagenicity study of potentially carcinogenic industrial compounds. Environmental Mutagen Society, New Orleans, Louisiana, March 8-12, 1979.

Ho, T., S. C. Tipton, and J. L. Epler. Induction of sister-chromatid exchanges (SCE) in human leukocytes by cyclic nitroso-compounds. Tissue Culture Association, Seattle, Washington, June 11-14, 1979.

Holland, J. M. Correlation between skin penetration, distribution and persistence of shale, coal and natural petroleum liquids and epidermal carcinogenicity in vivo. International Society for Occupational and Environmental Health, Park City, Utah, April 4-7, 1979.

Holland, J. Michael. Relative carcinogenicity: A strain independent measure of carcinogenic potency. Toxicology Speciality Group, Annual Meeting of the American College of Veterinary Pathologists, San Antonio, Texas, November 14-18, 1978.

Holland, J. M., L. H. Smith, and R. O. Rahn. Dosimetry of coal and shale derived crude liquids as mouse skin carcinogens. Symposium on Oil Shale Sampling, Analysis and Quality Assurance, Denver, Colorado, March 26-28, 1979.

Hsie, Abraham W., J. Patrick O'Neill, Juan R. San Sebastian, and Patricia A. Brimer. Quantitative mammalian cell mutagenesis and mutagen screening: study with CHO cells. NATO Advanced Research Institute, Monte Carlo, September 22-29, 1979.

Hsie, Abraham W., J. Patrick O'Neill, Juan R. San Sebastian, David B. Couch, Patricia A. Brimer, William N. C. Sun, James C. Fuscoe, Nancy L. Forbes, Richard Machanoff, James C. Riddle, and Mayphoon H. Hsie. Interrelationships of mutagenesis and carcinogenesis: Study of the mutagenicity of 101 agents using a quantitative mammalian cell mutation assay, CHO/HGPRT. XII International Cancer Congress, Buenos Aires, Argentina, October 5-11, 1978.

Huang, S. Mark and Wen K. Yang. In vitro DNA synthesis of isolated nuclei with endogenous and exogenous DNA polymerases: Studies on spleens of young and old Fischer 344 rats. Federation of American Societies for Experimental Biology (American Society of Biological Chemists), Dallas, Texas, April 1-10, 1979.

Huberman, E. and C. A. Jones. The uses of liver cell cultures in mutagenesis studies. Conference on Differentiation and Carcinogenesis in Liver Cell Cultures, New York Academy of Sciences, New York, October 9-12, 1979.

Huberman, Eliezer. Relationship between mutagenicity and transformation of mammalian cells in vitro by chemical carcinogens. XII International Cancer Congress, Buenos Aires, Argentina, October 5-11, 1978.

Huberman, Eliezer. Induction of terminal differentiation in human myeloid leukemia cells by tumor-promoting agents. American Association for Cancer Research, New Orleans, Louisiana, May 16-19, 1979.

Hwang, David L. and Wen K. Yang. Transfer RNA-primed in vitro reverse transcription of 28S ribosomal RNA. XIth International Congress of Biochemistry, Toronto, Canada, July 8-13, 1979.

Jacobson, K. Bruce, L. Opresko, and R. K. Owenby. Dietary cadmium alters transfer RNA isoacceptors in Drosophila. XIth International Congress of Biochemistry, Toronto, Canada, July 8-13, 1979.

Jagadeeswaran, P. and G. Lavelle. In vitro replication of single-stranded DNA of Kilham rat virus. Federation of American Societies for Experimental Biology (American Society of Biological Chemists), Dallas, Texas, April 1-10, 1979.

Jamasbi, R. J. Immunosuppression during the early phase of carcinogenesis enhances tumor development. 12th Annual Southeastern Immunology Conference, Mobile, Alabama, February 20-22, 1980.

Jamasbi, R. J. and E. H. Perkins. Immunological characterization of diethylnitrosamine (DEN) induced murine forestomach squamous cell carcinomas. American Association for Cancer Research, San Diego California, May 28-31, 1980.

Johnson, N. P., J. D. Hoeschele, and R. O. Rahn. Mechanism for the binding of cis- and trans-dichlorodiammine Pt(II) (DDP) to DNA. Biophysical Society, Atlanta, Georgia, February 25-28, 1979.

Johnson, Neil P., James D. Hoeschele, Ronald O. Rahn, and A. W. Hsie. Mutagenicity, cytotoxicity and DNA-binding of Pt(II)chloroammines in CHO cells. Environmental Mutagen Society, New Orleans, Louisiana, March 8-12, 1979.

Jones, Carol A., Virginia C. Dunkel, and Eliezer Huberman. The relationship between metabolism of nitrosamines to mutagens for mammalian cells and liver carcinogenesis. American Association for Cancer Research, San Diego, California, May 28-31, 1980.

Joshi, J. G. and P. A. Swenson. Disappearance of an inhibitor of malate dehydrogenase in E. coli after FAR-UV radiation. American Society for Photobiology, Carmel, California, June 24-28, 1979.

Kehrer, J. P., W. Haschek, and H. P. Witschi. Peracute toxicity of paraquat and diquat in 100% oxygen. Society of Toxicology, New Orleans, Louisiana, March 9-15, 1979.

Kehrer, James. P. and H. P. Witschi. The role of metabolism in the pulmonary toxicity of butylated hydroxytoluene (BHT). Federation of American Societies for Experimental Biology (American Society for Pharmacology and Experimental Therapeutics), Dallas, Texas, April 1-10, 1979.

Kehrer, James P. and H. P. Witschi. In vivo collagen synthesis in an experimental model of pulmonary fibrosis. Federation of American Societies for Experimental Biology, Anaheim, California, April 13-18, 1980.

Kimball, R. F. A two-lesion hypothesis for mutation induction in Haemophilus influenzae by N-methyl-N'-nitro-N-nitrosoguanidine. Environmental Mutagen Society, Nashville, Tennessee, March 17-20, 1980.

Klann, Richard C. and Ann C. Marchok. The effect of vitamin A on keratinization in tracheal epithelial cell lines. Southeastern Regional Meeting of the Society of Developmental Biology, Fontana Village, North Carolina, April 5-7, 1979.

Klein-Szanto, A. and P. Nettesheim. Different distribution frequency and ultrastructure of dark cells in carcinogen- and non-carcinogen-induced squamous metaplasias of the trachea. American Association for Cancer Research, San Diego, California, May 28-31, 1980.

Kootstra, A., M. C. Cronen, D. E. Olins, and T. J. Slaga. N-terminal fragments of histones remain associated with nucleosomes after tryptic digestion. 5th European Molecular Biology Organization Annual Symposium on Nucleic Acids Interactions, Heidelberg, Germany, October 12-15, 1979.

Kootstra, A. and T. J. Slaga. Implications of the binding of 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene to chromosomal histone proteins. 4th International Symposium on Polynuclear Aromatic Hydrocarbons, Columbus, Ohio, October 2-6, 1979.

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F. Financial Summary and Personnel Distribution

FY 1980

Funding Source	Funding in thousands	% of total budget	Person-years
Department of Energy	11,693	57.4	187.5
Environmental Protection Agency	4,733	23.2	78.0
National Cancer Institute	2,350	11.5	49.0
Department of Defense	500	2.5	5.7
National Institute of Environmental Health Sciences	400	2.0	9.5
National Aeronautics and Space Administration	98	0.5	0.7
Department of Agriculture	101	0.5	1.1
National Institute of General Medical Sciences	60	0.3	1.0
Food and Drug Administration	160	0.8	2.3
Miscellaneous	287	1.4	4.4
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