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Annual Progress Report

Period Ending September 30, 1978

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For Period Ending September 30, 1978

J. B. Storer, Director
T. T. Odell, Associate Director

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INTRODUCTION

The last Biology Division Annual Progress Report covered the period ending June 30, 1976. No report was issued in 1977 because there were serious doubts as to whether the reports were of sufficient value to justify the diversion of manpower and resources from scientific pursuits to their preparation and publication. In 1978 it became Laboratory policy to require annual reports from all research divisions, although not necessarily on an annual basis.

The period covered by the present report is October 1, 1977, through September 30, 1978. To give an indication of the work performed during the "missing" period July 1, 1976, through September 30, 1977, we have provided a listing of manuscripts published during this period. More detailed information may be obtained from our Reprint Selection Lists for 1977 and 1978.

Those readers familiar with our earlier annual reports will find the content and format of this one drastically different. Rather than describe work currently in progress, we have provided abstracts of manuscripts processed through our editorial office for the period October 1, 1977, through September 30, 1978. These abstracts should provide an adequate summary of research completed in this interval. They should not, of course, be cited without permission of the author(s). Many of these papers will have been published by the time this report is distributed; in cases where the reader has a particular interest in the work, it might be useful to contact the author(s) directly to ascertain the status of the manuscript.

In addition to the abstracts of papers, there are brief overviews of research activities of each of the sections. These overviews, in the aggregate, provide a divisional overview which will not be repeated here.

In keeping with scientific traditions, members of the Division disseminate their current research findings at various scientific meetings. A list of abstracts submitted for national and international meetings as well as a listing of additional presentations is provided.

The Biology Division has an active seminar program that features outstanding scientists from around the world. We have provided a list of speakers and seminar titles for the period October 1, 1977, through September 30, 1978.

We would appreciate comments from our readers on our new format and on how improvements might be made.

John B. Storer
Director, Biology Division

MUTAGENESIS AND TERATOGENESIS SECTION

The Mutagenesis and Teratogenesis (M & T) Section had a very active year, evidenced not only by its record of publications, outside lectures, and participation in national and international committees, but by the expansion of its activities, both in the basic and more programmatic areas. Three young senior investigators were added to the staff and a new program, Teratogenesis, was established. This year the Section organized the Division's annual symposium, and the subject chosen – Genetic Mosaics and Chimeras in Mammals – exemplifies the Section's interest in both genetic and developmental areas of research and their interface.

In the *in vivo* mammalian mutagenesis work, the Section has continued to provide an integrated approach to the study of chromosome damage (dominant lethals, heritable translocations, sex-chromosome loss), point mutations (somatic and transmitted specific-locus mutations), and indirect indicators of genetic damage (unscheduled DNA synthesis). Newer procedures, such as the "spot test" and dominants having skeletal effects, were explored for their potential as test systems.

The more programmatic portions of the whole-mammal work continued to provide the raw material for basic investigations. For example, the detailed genetic, cytogenetic, embryological, and biochemical study of over 100 mutants involving one of the loci screened for in specific-locus tests led to a new understanding of the nature of mammalian genes. In the course of this work a new regulatory system was discovered which has the potential of shedding unprecedented light on gene action in mammals.

In another area of basic interest, it was discovered this year that the mouse egg has a major repair system capable of dealing with lesions introduced by the male genome. Some genotypes are clearly repair deficient.

New approaches to the study of the mammalian gonads, both testes and ovaries, have improved our understanding of mutagenic effects. Most important was a study of the timing of oocyte development, which showed that the stage at which a radical shift in mutation frequency occurs in the mouse is comparable in all mammals so far studied.

In work with lower organisms and mammalian cells, two major systems for detecting the mutagenic effects of chemicals have been brought to good operating efficiency during the past year. The Comparative Mutagenesis program, in collaboration with the Analytical Chemistry Division, developed a combined chemical and biological approach to aid in identifying potentially hazardous substances in complex mixtures and began the application of this procedure to a number of materials associated with fossil fuel technology. The Mammalian Somatic Genetics program established the conditions for definitive tests for mutation induction using the *hgprt* locus in CHO cells. This methodology was tested with a variety of known mutagens and carcinogens, and a start has been made in applying it to detecting mutagens in materials associated with fossil fuel technology.

A number of studies with mammalian cells dealt with the processes involved in the repair of damage to DNA and the conversion of that damage to mutational changes, especially chromosomal aberrations. Investigation of repair in cultured human cells concerned the factors influencing the rate and completeness of repair. Studies with inhibitors of repair are opening up new ways to analyze the fate of DNA damage in human and other mammalian cells. Studies on chromosomal aberration induction by ionizing radiation and

chemicals in various types of mammalian cells, including germ cells, are yielding further information on the factors that influence the frequency with which aberrations are induced and detected.

The Mammalian Biochemical Genetics program has been developing methods to detect mutations and other error-producing events that result in proteins with altered amino acid sequences. During the year several different proteins were examined for variants.

The *Drosophila* Genetics and Cytology program obtained additional and strong evidence that recombination occurs in premeiotic interphase at the time that DNA synthesis is also occurring. This is considerably earlier than had been widely believed. The finding leads to new views about the recombination process and about the events that can affect chromosome distribution at meiosis.

Work with bacteria in the Section emphasized DNA repair processes and the relation between mutation induction and DNA repair and replication. This work should help to elucidate the repair and mutation processes in general and is basic to a critical evaluation of the pertinence to human hazards of the bacterial test systems that are now widely used as first screens for mutagens and carcinogens.

While the new mouse teratogenesis program was getting under way, an earlier *in vivo* approach utilizing quantitative (homeotic) shifts in minor skeletal characters was developed into a sensitive teratological prescreen.

Teratogenic end points in amphibians were used by the Development and Reproductive Physiology Unit to test for adverse effects of various substances. In addition, lower aquatic organisms (*Tetrahymena, Daphnia*) were employed in tests of products of various emerging fossil fuel technologies.

Among several important breakthroughs achieved this year in the study of invertebrate oocytes was the development of a method for long-term culture of *Xenopus* oocytes, without accessory cells, which will allow study of mechanisms regulating growth and differentiation.

Induction of Specific-Locus Mutations in the Mouse by Tritiated Water

W. L. Russell, R. B. Cumming, E. M. Kelly, and E. L. Phipps

The results reported are the first obtained on transmitted gene mutations induced by tritium in any form in any mammal. They are, therefore, of obvious practical importance in the estimation of the possible biological hazards of man-made tritium in the environment. Male mice were injected intraperitoneally with either 0.75 or 0.50 mCi per gram of body weight of tritiated water. They were then used in our standard specific-locus mutation test in which the treated wild-type stock of mice is mated to a stock homozygous for seven recessive marker genes. Mutations at any of the seven loci are scored in the offspring. The earlier matings provided information on the mutation frequency in germ cells irradiated in postspermatogonial stages, and the later matings gave the mutation frequency in treated spermatogonia. The spermatogonia are the important cells so far as human risks are concerned, and the mouse results for this germ-cell stage yielded a relative biological effectiveness of approximately 2 for tritiated water compared with low-dose-rate gamma irradiation. There are various uncertainties involved in arriving at this figure, and the difference between it and 1 is probably not statistically significant. However, for risk estimation, it seems prudent to use the relative biological effectiveness value of 2, which is, after all, the best point estimate computed from the present data.

Mutational Repair in Mammals and Its Bearing on Risk Assessment

W. L. Russell

Abstract not available.

Induction of Dominant Mutations that Cause Skeletal Malformations in Mice

Paul B. Selby

A new approach for estimating genetic risk to humans from radiation is based on an analysis of the frequency of induction of dominant mutations that cause skeletal abnormalities in mice. The main goal of this work is to improve estimates of the effect that an increase in the mutation frequency would have upon the incidence of serious genetic diseases in humans. The data obtained relate to dominant and irregularly inherited conditions in humans, which together constitute the great majority of human genetic diseases. The skeletal method could be used in chemical mutagenesis research in order to make a much more accurate risk-benefit analysis. A more likely application, however, is to provide a relatively quick and easy mammalian testing procedure for identifying mutagens. Dominant mutations at an unknown, but probably large, number of genetic loci could be detected. The relatively quick and easy procedure, which is described, has not yet been tested.

Repair in the Egg of Chemically Induced Genetic Lesions in Spermatozoa and Spermatids of Mice

Walderico M. Generoso, Katherine T. Cain, Maryala Krishna, and Sandra W. Huff

Conclusive proof that the mouse egg is capable of carrying out repair of genetic lesions present in the male genome was obtained through dominant-lethal studies of chemically treated spermatozoa and spermatids and through cytological analysis of first-cleavage metaphases. The maximum difference in repair capability between stocks of females, found for isopropyl methanesulfonate treatment, was large; considerably smaller differences were found for ethyl methanesulfonate, triethylenemelamine, and benzo[*a*]pyrene treatments. No difference was found for X-ray treatment.

Ethylation of DNA and Protamine by Ethyl Methanesulfonate in the Germ Cells of Male Mice and the Relevancy of These Molecular Targets to the Induction of Dominant Lethals

Gary A. Sega and J. G. Owens

The molecular dosimetry of ethyl methanesulfonate (EMS) in the germ cells of male mice has been investigated. The mice were injected intraperitoneally with 200 mg/kg of [^{3}H]EMS and the ethylations per sperm head, per deoxynucleotide, and per unit of protamine were then determined over a 2-week period. The ethylations per sperm head closely paralleled the dominant-lethal frequency curve for EMS, reaching a maximum of 5 to 6.5 million ethylations per vas sperm head at 8 to 10 days after treatment. Ethylation of sperm DNA was greatest at 4 hr after treatment, with 5.7 ethylations per 10^5 deoxynucleotides, and gradually decreased to 2.2 ethylations per 10^5 deoxynucleotides at 15 days after treatment. The ethylation of sperm DNA did not increase in the germ-cell stages most sensitive to EMS and was not correlated with the dominant-lethal frequency curve for EMS. However, ethylation of sperm protamine did increase in the germ-cell stages most sensitive to EMS and showed an excellent correlation with the incidence of dominant lethals produced by EMS in the germ cells.

A model is presented to explain, at a molecular level, how dominant lethals may be induced in mouse germ cells by EMS. Ethylation of cysteine sulfhydryl groups contained in mouse sperm protamine could block normal disulfide-bond formation, preventing proper chromatin condensation in the sperm nucleus. Stresses in the chromatin structure could then eventually lead to chromosome breakage, with resultant dominant lethality.

Somatic Cells as Indicators of Germinal Mutations in the Mouse

Liane B. Russell

In attempts to find a prescreen for mutagens that may induce heritable mutations in mammals, an *in vivo* somatic mutation test has been developed in the mouse that uses a localized gene product (hair pigment), is relatively fast and inexpensive, and gives results that have some predictive value for point mutation induction in spermatogonia. Embryos heterozygous at specific coat color loci are exposed to the presumptive mutagen, and 3 weeks later the fur is observed for spots of altered color. It is possible to distinguish spots resulting from expression of the recessive (RSs) from spots having various other causes.

In tests with seven compounds, mutation rates per locus and unit dose have been calculated on the assumption that 175 cells are at risk per $10\frac{1}{4}$-day embryo (a number derived from distribution of spot proportions). These rates are found to be roughly parallel to, but uniformly higher than, spermatogonial point-mutation rates for the same seven compounds. The higher somatic rates are presumably due to the fact that RSs can result from several genetic mechanisms besides point mutations. The spot test, which has not to date given any false negatives, may thus be considered a useful *in vivo* prescreen for heritable germinal mutations in mammals.

Analysis of the Albino-Locus Region of the Mouse. I. Origin and Viability

Liane B. Russell, W. L. Russell, and E. M. Kelly

Numerous specific-locus experiments designed to test the mutagenic effect of external radiation have yielded, in over 3,600,000 animals observed, altogether 119 presumed mutations involving the *c* locus. Of these, 55 were viable and albino ($c^{a\nu}$), 13 were viable and of various intermediate pigment types ($c^{x\nu}$), 4 were subvital (c^{as} and c^{xs}), 7 were neonatally lethal albinos ($c^{a\ell}$), 28 were prenatally lethal albinos ($c^{a\ell}$), and 12 died untested. All of the prenatally lethal and at least one of the neonatally lethal *c*-locus mutations ($c^{a\ell}$ classes) are probably deficiencies that we have analyzed more extensively in other experiments. Since absence of the locus mimics *albino* in phenotype, the intermediates ($c^{x\nu}$ and c^{xs} groups) probably resulted from intragenic changes. The class of viable albino mutants ($c^{a\nu}$) may include, in addition to intragenic changes, some extremely small deficiencies. The effects on viability of *c*-locus lethals ($c^{a\ell}$s) in heterozygous condition are not drastic enough to be perceived in stocks of mixed genetic background except in the case of the two longest known deficiencies and a few others.

Analysis of the relation between radiation treatment and type of *c*-locus mutants obtained shows that the relative frequency of viable mutations, for each germ cell type, is greater for low linear energy transfer (LET) than for neutron irradiation; however, the difference for any individual cell type is not significant. The majority (66.7%) of mutations derived from X- or γ-ray irradiated spermatogonia are viable, and the proportion of "intermediates" among these viables is similar to that among presumed spontaneous *c*-locus mutations. No significant dose-rate effect on the proportion of lethals could be demonstrated within the set of mutants induced by low-LET irradiation of spermatogonia. Although sets from other germ cell stages are too small for statistical tests, the results for oocytes are similar, as far as they go. Furthermore, most of the *c*-locus mutations induced in spermatogonia, even by high-dose-rate X- or γ-irradiation, are of a type most likely to result from single-track events (62% $c^{x\nu}$, c^{xs}, and $c^{a\nu}$; plus 16% presumed deficiencies not involving closest marker). These results support the view that most of the reduction in mutation frequency at low dose rates is not due to a change in relative proportion of 2-track and 1-track ionizing events.

Analysis of the Albino-Locus Region of the Mouse. II. Mosaic Mutants

Liane B. Russell

Among 119 mutations involving the *c* locus that were recovered in the course of mouse specific-locus experiments with external radiations, 16 were found in mosaic, or fractional, mutants. The number of

additional *c*-locus fractionals that could have occurred in these experiments, for a variety of reasons, might not have been clearly identified, probably does not exceed the present number. There was no evidence for radiation induction of the fractionals, and even those occurring in the irradiated groups may thus be assumed to be of spontaneous origin. Since only two mutations in the control groups were found in whole-body mutants, it appears that the bulk of spontaneous *c*-locus mutations are fractionals. None of the mutations recovered in fractional mutants was homozygous lethal; 25% were viable intermediate alleles, and the remainder were albino-like mutants, all viable except for one subvital and one not tested. Genetic tests of the fractionals indicated no major selection against the new mutations, either gametically or in the progeny.

For the group of fractionals as a whole, about one-half of the germinal tissue carried the mutation, indicating that the fractionals came from an overall blastomere population that was one-half mutant. Such a population could result from mutation in one strand of the gamete DNA, in a daughter chromosome derived from pronuclear DNA synthesis of the zygote, or in one of the first two blastomeres prior to replication. Since the mouse embryo does not stem from all of the cleavage products of the zygote, the frequency of fractionals observed underestimates the frequency of mutational events that result in two types of blastomeres.

Analysis of the Albino-Locus Region of the Mouse. III. Time of Death of Prenatal Lethals

Liane B. Russell and G. D. Raymer

The stage at which homozygotes die was determined for 28 mutations (general symbol c^*) at the *albino* (*c*) locus, of which 26 had earlier been found to be probably prenatally lethal. Within each of the mutant stocks, the uterine contents of c^*/c^{ch} females, made pregnant either by c^*/c^{ch} males ("Ex" series) or by c^{ch}/c^{ch} males ("Co" series), were examined between 13 and 17 days postconception. Altogether, 743 females were dissected and 7197 corpora lutea (representing ovulations) counted. In selected stocks, an additional 40 and 13 females were dissected on days 7 or 9 respectively. In each of the 26 presumed prenatally lethal mutants, there was a deficiency of living fetuses in the Ex, as compared with the Co, group. Overall, this deficiency was 23.6% (expectation, 25% c^*/c^*). All meaningful excesses were in numbers either of moles (death shortly before, during, or just after implantation), or of early preimplantation losses. Homozygotes in none of the mutant stocks die between days 9 and 19 postconception. Of 24 *c*-locus mutants known to be deficiencies since they lack the closely linked *Mod-2*, 13 kill clearly before implantation, 10 around implantation, and one neonatally. The *c* and *Mod-2* loci and the region between them are not needed for intrauterine survival.

There are indications that the distinction between early-preimplantation death and implantation death may, in a general way, be related to length of the deficiency.

Sensitivity Patterns for the Induction of Homeotic Shifts in a Favorable Strain of Mice

Liane B. Russell

In an effort to develop a prescreening system that is sensitive, is compatible with *in vivo* administration of presumptive teratogens, and utilizes morphological end points, we are studying the induction of homeotic shifts in the axial skeleton. Our earlier work demonstrated the existence of critical periods for certain such shifts, even though the (C57BL X NB)F_1 hybrid used was not entirely suitable for this purpose. Using 100-R X rays on successive days during the period of major organogenesis, we analyzed the sensitivity pattern of the BALB/c, since this strain normally straddles thresholds for the position of axial borders. Mortality, birth weight, and skeletal anomalies were analyzed, in addition to the homeotic shifts. Day $9\frac{1}{4}$ postconception was clearly the most sensitive stage for the production of posterior shifts at the thoraco-lumbar border and the lumbo-sacral border, and for increases in the number of sternebrae and costo-sternal

junctions. Anterior shift could be induced 3 days later, on day 12¼, but this was confined to only one of the four characters, the thoraco-lumbar border. There are a number of indications that, at given stages during the study period, BALB/c embryos correspond developmentally to chronologically somewhat earlier stages of two strains studied previously. The present results do not support the hypothesis that factors influencing the quantitative skeletal characters act by way of body size. Since homeotic shifts can be produced by a number of mechanisms and are obvious indications that developmental interference has occurred and since such shifts are easy to score and convenient to analyze quantitatively, our identification of a highly suitable strain and of stages of maximum sensitivity in this strain should aid in making this system useful in prescreening for chemical teratogens.

Application of a Sensitive *In Vivo* Teratological System to the Testing of Benzo[*a*]pyrene

Liane B. Russell and T. W. McKinley, Jr.

Effects on morphogenesis from benzo[*a*]pyrene (BP) injections to pregnant females have, for the first time, been demonstrated by (*a*) choosing embryonic stages shown earlier to have maximum sensitivity for the induction of homeotic shifts and (*b*) choosing a mouse strain, BALB/c, favorable for revealing such shifts and genetically responsive to the induction of aryl hydrocarbon hydroxylase activity. BP administered 18 hr prior to a stage of maximum sensitivity, as determined with X rays, produced qualitatively the results typical of this sensitive period for each of four homeotic characters (shifts in positions of vertebral borders and in sternal features) and for birth-weight reduction. These results indicate that the BP-induced disturbance in the embryo was not delayed for more than a day after injection, but further experiments are needed to determine the earliest onset of this disturbance. The effects increased with dose and were observable in all homeotic characters at the lowest dose used, 50 mg of BP per kilogram of body weight given intraperitoneally. In addition to the clear-cut action on quantitative characters, BP also produced limited mortality and some outright abnormalities, a few of which were externally recognizable. However, injections of 50 or 100 mg of BP per kilogram of body weight were considerably less effective than were 100-R X rays, administered at the corresponding stage, in inducing such morphological abnormalities. Even though the quantitative shifts, too, were induced at a lower frequency by the BP injections than by 100 R, these shifts were so clear-cut as to confirm the usefulness of homeotic characters in the discovery of weak teratogenic effects.

The Use of Mouse X-Autosome Translocations in the Study of X-Inactivation Pathways and Nonrandomness

Liane B. Russell and Nestor L. A. Cacheiro

By means of autoradiographic study of an array of mouse X-autosome translocations with different breakpoints, we have 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, 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.

Genetic Mosaics and Chimeras in Mammals

Liane B. Russell

The book contains 31 chapters by investigators engaged in mosaic and chimera research in mouse and man. Organization is by problem area addressed, rather than by mode of origin of the mosaic condition. Subjects include the use of chimeras and mutational or disjunctional mosaics to study gamete differentiation, sexual development, and origin of the germline; the use of chimeras to study developmental potency, gene expression, control of phenotype, and tumor origin; X-chromosome inactivation and derepression; X-chromosome nonrandomness (imprinting and selection); and mathematical analysis of mosaic patterns.

Genetic Mosaics and Chimeras in Mammals – Preface and Summary

Liane B. Russell

The study of mosaicism – the coexistence in the same individual of different functional genotypes – forms a highly active interface between the fields of genetics, experimental embryology, and cell biology. The past few years have seen explosive development in the field of mammalian mosaicism, both natural (as it exists, for example, in female mammals due to random inactivation of one X chromosome) and artificial (as produced by the operative combination of components of separate embryos to form chimeras). This rapid development has its basis in recent advances in embryological microsurgery, cytological aids (such as chromosome banding and labeling), cell hybridization, and mammalian genetics, which has discovered and characterized useful biochemical markers, sex-reversal genes, and valuable chromosome translocations. A highly exciting component of the new chimera work is the use of teratocarcinoma systems that have been shown capable both of normal development and malignancy.

Mouse Mitochondrial Malic Enzyme: Purification and Differences in Expression in Brain Between Inbred Strains

Edward G. Bernstine

A mitochondrial malic enzyme [EC 1.1.1.40; L-malate:$NADP^+$ oxidoreductase (oxaloacetate-decarboxylating)] has been extensively purified from mouse heart and brain and partially characterized. The purified preparations showed one major polypeptide band on 10% sodium dodecylsulfate–polyacrylamide gels of molecular weight 62,000. The molecular weight of the enzyme was determined in the analytical ultracentrifuge to be 250,000–253,000, thus providing strong evidence that the native protein is a tetramer whose subunits have identical molecular weights.

Rabbit antiserum has been prepared against the purified heart enzyme. The antiserum is specific for the mitochondrial form of malic enzyme and cross-reacts completely with the enzyme from brain. Using this antiserum, it has been shown that a tissue-specific difference in the specific activity of the enzyme in brains of two inbred strains of mice is due to the presence of more enzyme molecules in one of the strains than in the other.

Regulation of Mitochondrial Malic Enzyme Expression in Mouse Brain

Edward G. Bernstine, Chongkun Koh, and Carolyn Cain Lovelace

A developmental study has shown that the adult level of mitochondrial malic enzyme in mouse brain is attained within the first 8 weeks after birth. Inbred strains possessing high activity are characterized by a more rapid rate of increase of enzyme activity and a higher adult level than low-activity strains. C3H and C57BL/10 mice were chosen as model high- and low-activity strains, respectively, for genetic and biochemical analyses. Examination of backcross progeny showed that the factor determining the difference in brain activity levels segregates as a single genetic locus which has been named *Mdr*. The *Mdr* locus is located on mouse chromosome 7; it is approximately the same distance from *Hbb* as the structural gene for mitochondrial malic enzyme (*Mod-2*). Pulse-labeling experiments have shown that *Mdr* controls the rate of synthesis of mitchondrial malic enzyme in the brain.

Genetics of Formamidase-5 (Brain Formamidase) in the Mouse: Localization of the Structural Gene on Chromosome 14*

R. B. Cumming, Marva F. Walton, J. C. Fuscoe,† B. A. Taylor,‡
J. E. Womack,‡§ and F. H. Gaertner

A single formamidase, which is different from the formamidases found in other tissues, occurs in the brains of mice. This enzyme is here called formamidase-5, and the gene symbol is designated *For-5*. Two alleles are recognized on the basis of their differential heat sensitivity; $For\text{-}5^b$ is relatively heat stable and is present in strain C57BL/6J, whereas $For\text{-}5^d$ is relatively heat sensitive and is present in strain DBA/2J. The heat sensitivity of formamidase-5 in 44 other inbred strains and substrains was tested and found to resemble that of C57BL/6J or DBA/2J. Thirty-six recombinant inbred strains derived from progenitors that differed at *For-5* were studied to test for single-gene inheritance and linkage with other loci. Complete concordance was found with the esterase-10 locus (*Es-10*), indicating close linkage. The 99% upper confidence limit of the distance between *For-5* and *Es-10* is 3.7 centimorgans (cM). *Es-10* is located on chromosome 14 about 19 cM from the centromere. An independent demonstration of linkage of *For-5* with *Es-10* and another chromosome 14 marker, hairless (*hr*), is provided by the finding that the HRS/J strain, which has been sibmated for 60 generations with forced heterozygosity at the *hr* locus, is co-segregating at *For-5* and *Es-10*. A survey of 32 inbred strains and substrains revealed that the $For\text{-}5^d$ allele is associated with the $Es\text{-}10^b$ allele and that the $For\text{-}5^b$ allele is associated with $Es\text{-}10^a$ and $Es\text{-}10^c$. Formamidase-5 segregates as expected in the F_2 generation of crosses between strains bearing $For\text{-}5^b$ and $For\text{-}5^d$ alleles. It is possible that this unique formamidase of the brain is involved in the metabolism of a neurotransmitter substance.

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†Predoctoral investigator, Grant CA 09104 from the National Cancer Institute.

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Timing of Oocyte Maturation in the Mouse and Its Relevance to Radiation-Induced Cell Killing and Mutational Sensitivity

E. F. Oakberg

Timing oocyte development by labeling the zona pellucida indicates that it takes 6 weeks (possibly a few days more) for a stage 3b oocyte to reach ovulation. Thus the shift in mutation frequency with time after irradiation occurs in an oocyte stage that is comparable in all mammals so far investigated, and in the mouse low-mutational sensitivity is not restricted to the arrested dictyate oocyte stage. Some oocytes with nuclear morphology similar to the arrested human oocytes give low mutation rates. Degree of chromosome condensation in early oocytes does not appear to be a reliable criterion of oocyte sensitivity to either cell killing or mutation induction, and genetic data on mouse oocytes may be more generally applicable than commonly thought.

Follicular Growth and Atresia in the Mouse

E. F. Oakberg

Follicles were classified on the basis of number of layers of follicle cells, presence and degree of development of the zona pellucida, and presence of an antrum. Formation of an antrum in follicles with less than 7–8 cell layers and/or presence of necrotic cells was considered indicative of degeneration. When classified in this manner, the data suggest that follicles and their contained oocytes are committed to either normal development or atresia by the time a third layer of granulosa cells is formed.

Morphological and Quantitative Analysis of Spermatogonia in Mouse Testis Using Whole-Mounted Seminiferous Tubules. I. The Normal Testes

C. Huckins* and E. F. Oakberg

The spermatogonial populations in ten normal adult mice were analyzed using whole-mounted seminiferous tubules. The undifferentiated A spermatogonia as well as the six generations of differentiating spermatogonia were clearly identifiable on whole mounts. Description plus quantitation of these cell types revealed that they behaved in essentially the same manner as their counterparts in the rat. Single undifferentiated A cells were classified as type A_s stem cell spermatogonia. They were distributed throughout the seminiferous epithelium and by periodic mitoses maintained their stock and furnished cells which would eventually differentiate. Initially, the latter morphologically resembled the A_s spermatogonia. However, they were classified as type A_{al} spermatogonia because they were linked by cytoplasmic bridges and because they usually underwent one or more synchronous mitotic divisions to form short chains of aligned cells. Ultimately, division of these cells ceased, and they assumed the characteristics of A_1 spermatogonia which continued to differentiate according to the well-established pattern. The cyclic production of cohorts of A_1 cells by the stem cell population ensured a continual supply of spermatogonia for differentiation.

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Morphological and Quantitative Analysis of Spermatogonia in Mouse Testis Using Whole-Mounted Seminiferous Tubules. II. The Irradiated Testes

C. Huckins* and E. F. Oakberg

In adult male mice exposed to 300-R X-irradiation, the spermatogonial population was selectively killed except for the radioresistant type A_s stem cells. Type A spermatogonia were minimal 2 days after irradiation, when only 20% of the control population was present in stages 5–6. When multiple injections of ^{3}HTdR were given between 2 and 3.5 days postirradiation, 90–95% of these survivors in stages 4–6 became labeled, preparatory to initiating repopulation of the epithelium. Enhanced proliferation by these stem cells and at times when they were normally quiescent led to restoration of all classes of spermatogonia by 11 days after irradiation.

Several autoradiographic studies were undertaken to better characterize the radioresistant cells. In mice given single or multiple injections of ^{3}HTdR before irradiation, there was appreciable retention of label by those type A_s spermatogonia that had incorporated ^{3}HTdR in stages 2–4. This labeling pattern was coincident with that of the long-cycling A_s stem cells in nonirradiated testes. Since the long-cycling A_s stem cells are characterized by a prolonged G_1 or "A-phase," which is known to be a highly radioresistant portion of the cell cycle, these cells preferentially survive irradiation doses that kill other spermatogonial types. It is proposed that following germ cell depletion, as in irradiation injury, the long-cycling A_s survivors would all be prematurely triggered from A-phase to begin DNA synthesis and initiate restoration of the germ cell population.

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The Use of Short-Term Tests in the Isolation and Identification of Chemical Mutagens in Complex Mixtures*

J. L. Epler

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 (*a*) predictors of profound long-range health effects such as mutagenesis and/or carcinogenesis, (*b*) a mechanism to rapidly isolate and identify a hazardous biological agent in a complex mixture, and (*c*) a measure of biological activity, correlating base-line 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, the investigator can accumulate information on the actual compounds responsible for the biological effect. Thus, the mutagenicity tests will also 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.

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Energy-Related Pollutants in the Environment: The Use of Short-Term Tests for Mutagenicity in the Isolation and Identification of Biohazards

J. L. Epler, F. W. Larimer, T. K. Rao, C. E. Nix, and T. Ho

In an effort to gather information on the potential genetic hazards of existing or proposed energy-generating or -conversion systems, we have begun a correlated analytical and genetic analysis of a number of technologies. The work is divided into two phases: one deals with known compounds expected to occur in the environment through energy production, conversion, or use; the other deals with actual samples from existing or experimental processes. To approach the problems of coping with and testing large numbers of compounds, we set up a form of the "tier system." Operating units utilizing *Salmonella, Escherichia coli*, yeast, human leukocytes, mammalian cells, and *Drosophila* have been initiated. Various liquid-liquid extraction methods and column chromatographic separations have been applied to crude products and effluents from oil-shale, coal-liquefaction, and coal-gasification processes. Mutagenicity of the various fractions is assayed by means of reversion of histidine-requiring auxotrophs of *Salmonella typhimurium*; comparative studies are carried out with the other genetic systems. In order to incorporate metabolic activation of these fractions and compounds, rat liver homogenates (S-9) are used in the various assays. Results implicate chemicals occurring in the basic (ether-soluble) and the neutral fractions as potential genetic hazards. Chemical constituents of these fractions (identified or predicted) were tested individually for their mutagenic activity.

Mutagenicity Testing of Energy-Related Compounds*

J. L. Epler

Abstract not available.

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Mutagenic Components of Alternate Energy Sources*

James L. Epler and Michael R. Guerin†

Abstract not available.

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†Analytical Chemistry Division, Oak Ridge National Laboratory.

Short-Term Bioassay of Complex Organic Mixtures: Part I. Chemistry*

M. R. Guerin,† B. R. Clark,† C.-h. Ho,† J. L. Epler, and T. K. Rao

Petroleum substitutes produced from coal and shale are among the materials requiring biological evaluation to assess environmental and health impacts of new energy technologies. Intractability and the presence of highly toxic constituents are among the physical and chemical properties of products and process streams which complicate short-term biotesting for subtle health effects. An effective approach is to separate the starting material into chemically well-defined fractions. Bioassay results obtained for the separated fractions may be summed to estimate the biological activity of the starting material. Biological activities of individual fractions provide evidence as to the types of constituents responsible for the biological activity of the starting material. Chemical fractionation, quality control, and the identification of bioactive species are the roles of chemists in supporting short-term bioassays.

Isolates of selected classes of chemicals may be prepared for bioassay when the chemical class of importance is known. Alternatively, the starting material may be separated to preserve all of the original constituents in a small number of fractions for biotesting. Liquid-liquid partition from strongly acidic and alkaline solutions has proven viable for the testing of coal- and shale-derived oils. A theoretically more gentle separation procedure based on Sephadex LH-20 gel chromatography has been found viable for lipophilic materials.

Studies suggest that alkaline constituents of petroleum substitutes are major contributors to their Ames test activity. Subfractionation of ether-soluble bases from a shale- and coal-derived oil has concentrated the bioactive constituents in a fraction constituting approximately 0.5 wt % of the starting oil. Nitrogen heterocyclics are found to be the predominant constituents of this active subfraction.

*Research sponsored jointly by the Environmental Protection Agency and the Division of Biomedical and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with Union Carbide Corporation.

†Analytical Chemistry Division, Oak Ridge National Laboratory.

Short-Term Bioassay of Complex Organic Mixtures: Part II. Mutagenicity Testing*

J. L. Epler, B. R. Clark,† C.-h. Ho,† M. R. Guerin,† and T. K. Rao

Mutagenicity testing of fractionated complex organic mixtures represented by crude natural and synthetic oils, aqueous condensates, and process waters from liquefaction and shale oil technologies, along with leachates and organic extracts of raw materials, wastes, and particulate materials has been carried out. Preliminary screening with the *Salmonella* histidine reversion system utilizing both missense and frame-shift strains was applied to the test materials. Individual specific activities (revertants per mg of fraction) can be summed to yield an estimate of the mutagenic potential of the crude starting material. Total activity varies from process to process, but specific basic and neutral fractions consistently contained the bulk of the mutagenic activity. Metabolic activation with rat liver extracts is routinely required for activity, and

differentiation of fractions can be achieved by assay with variously induced enzyme preparations. The bacterial mutagenesis assays aid in isolating and identifying the actual components representing the genetic biohazard. Parallel testing of identified or predicted constituents was carried out.

Comparative and validative mutagenesis assays were performed with the *Escherichia coli* system of Mohn, the yeast system, the *Drosophila* sex-linked lethal assay, and mammalian cell cytogenetic tests. The short-term genetic tests can be considered as useful screens for potential biohazards of various effluents both in plant and in the immediate plant environment.

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†Analytical Chemistry Division, Oak Ridge National Laboratory.

Genetic Toxicity Testing of Complex Environmental Effluents*

F. W. Larimer and J. L. Epler

We have used the Ames *Salmonella* histidine-reversion system and other genetic test systems to assay the mutagenic potential of crude synthetic oils and natural crude oils as exemplary complex mixtures. Extracts and leachates from particulate matter are also considered. Mutagenicity data on isolated or suspected organic components are presented. The results support the use of the short-term genetic tests in examining crude mixtures and point to the advantages of coupling the bioassays with chemical fractionation.

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Separation of Mutagenic Components of Synthetic Crudes*

C.-h. Ho,† Bruce R. Clark,† Michael R. Guerin,† T. K. Rao, and James L. Epler

A basic alumina column eluted first with benzene and then ethanol isolates the mutagenic components of the ether-soluble base fractions (ESB) of synthetic crude oils into a fraction of about 25% (wt) of the ESB. A further separation is achieved by eluting the ethanol isolate through a Sephadex LH-20 gel column with isopropanol followed by acetone. About 90% of the mutagenic activity of the ESB is recovered in the acetone subfraction, which comprises ~0.5% (wt) of the crude oil. Development of this separation scheme was made possible by using the microbial mutagenesis assay to locate bioactive constitutents during exploratory liquid chromatographic separations.

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†Analytical Chemistry Division, Oak Ridge National Laboratory.

Mutagenicity of Fractionated Test Material from the Synthetic Fuel Technology with Bacterial Systems*

T. K. Rao, J. A. Young, A. A. Hardigree, W. Winton, and J. L. Epler

The predictive value of short-term genetic tests, such as the *Salmonella* and *Escherichia coli* (K-12, 343/113) systems including microsomal activation, is well documented. We have applied short-term testing to various crude products and effluents from the synthetic fuel technologies. Class fractionation and column chromatography of the test materials and the coupled bioassays can be used to identify the most active fractions (collaborative effort with Analytical Chemistry Division). Reversion at the histidine locus

for *Salmonella* was assayed with each fraction, and the results are expressed in units of revertants (strain TA98) per milligram of the starting material (organic content) including metabolic activation with a crude rat liver preparation. Results obtained with the *Salmonella* system were validated by employing *E. coli* strains auxotrophic for arginine. Genetic activity is seen with a variety of fractions, largely the basic and neutral (PAH) components. Total activity varies from process to process; thus the short-term genetic test can be considered a useful prescreen for potential biohazard of various effluents both in plants and in the immediate plant environment.

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Status of Bioscreening of Emissions and Effluents from Energy Technologies*

Michael D. Waters† and James L. Epler

Short-term bioassays are being applied effectively in the detection and evaluation of potentially hazardous emissions and effluents from conventional and developmental energy technologies. Biological screening tests such as the Ames *Salmonella*/microsome assay have demonstrated their utility (*a*) as indicators of potential long-term health effects such as mutagenesis and carcinogenesis, (*b*) as a means to direct the fractionation and identification of a hazardous biological agent in a complex mixture, (*c*) as a measure of relative biological activity to be correlated with changes in process conditions, and (*d*) to establish priorities for further confirmatory short-term bioassays, testing in whole animals, and more definitive chemical analysis and monitoring. The reliability of screening tests can be improved when they are combined with other short-term bioassays and employed in concert as a test battery. This is so because environmental agents may be endowed with specific kinds of biological activity such that they are detected in some systems but not in others. Current information suggests that the short-term test batteries for genotoxic effects should include, as a minimum, tests for point mutations, chromosomal aberrations, primary damage to DNA, oncogenic transformation *in vitro*, and toxicity related to each of these effects. Toxicity tests continue to rely heavily on conventional methodology.

A major achievement of the Interagency Energy/Environment R & D Program has been the development and implementation of short-term bioassays that are capable of detecting multiple biological activities. The attributes of several of these systems will be discussed in relation to their function within the test matrix. Biochemical techniques that have advanced the state of the art in short-term testing will be discussed.

Preliminary chemical fractionation and analysis is critical in the effective utilization of most bioassay techniques for complex sample evaluation, especially when toxicity is found to interfere with tests for genotoxic effects. The biological direction of chemical fractionation and analysis of environmental effluents and crude products from the synthetic fuels technology will be described as an example of the combined use of chemical and biological methodology. Details of the fractionation scheme and the results of microbial screening tests and comparative mutagenesis bioassays will be presented to emphasize the utility of the combined approach in energy-related research.

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Mutagenicity of Aliphatic Nitrosamines in *Salmonella typhimurium**

T. Kameswar Rao, J. A. Young, W. Lijinsky,† and J. L. Epler

Twenty-five aliphatic nitrosamines were examined in the Ames assay for bacterial mutagens using rat liver microsomes for activation. Of them, eight carcinogens were mutagenic, and five noncarcinogens were not

mutagenic. However, two noncarcinogens were mutagenic and nine carcinogens were not mutagenic, including five that are liver carcinogens in rats.

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Mutagenicity of Cyclic Nitrosamines in *Salmonella typhimurium:* Effect of Ring Size*

T. Kameswar Rao, Della W. Ramey, W. Lijinsky,† and J. L. Epler

Mutagenicity of cyclic nitrosamines with varying carcinogenic potentials was assayed in the *Salmonella* histidine reversion system. Mutagenicity in the pour-plate assay was compared with that in the liquid preincubation test. The smaller ring compounds (nitrosoazetidine, nitrosopyrrolidine, and nitrosopiperidine) exhibited a similar effect in both assays. The larger ring compounds (nitrosohexamethyleneimine, nitrosoheptamethyleneimine, nitrosooctamethyleneimine, and nitrosododecamethyleneimine) were more effective in the liquid preincubation test. Our results suggest a reasonable relationship between their mutagenic and carcinogenic activities.

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The Role of *Drosophila* in Chemical Mutagenesis Testing*

Carroll E. Nix and Bobbie Brewen

Abstract not available.

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Integrated Chemical and Biological Testing of Synthetic Oils and Effluents. Part I. Short-term mutagenicity testing*

J. L. Epler, C. E. Nix, T. Ho, F. W. Larimer, and T. K. Rao

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Phenotypic Expression Time of Mutagen-Induced 6-Thioguanine Resistance in Chinese Hamster Ovary Cells (CHO/HGPRT System)*

J. Patrick O'Neill and Abraham W. Hsie

Mutation induction at the hypoxanthine-guanine phosphoriboxyl transferase (HGPRT) locus in Chinese hamster ovary (CHO) cells (referred to as the CHO/HGPRT system) can be quantitated by selection for the phenotype of resistance to 6-thioguanine (TG) under stringently defined conditions. The phenotypic ex-

pression time, that is, the time interval after mutagen treatment which is necessary before all mutant cells are able to express the TG-resistant phenotype, has been found to be 7–9 days in this CHO/HGPRT system when the cells are subcultured every 48 hr. Subculture in medium with or without hypoxanthine (HX) utilizing trypsin, ethylenediaminetetraacetic acid (EDTA), or ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA) for cell removal yields identical results. When subculture at intervals greater than 48 hr is employed, a slight lengthening of the expression time is observed. An alternative method to regular subculture has also been achieved by maintaining the cells in a viable, nondividing state in serum-free medium. This procedure yields a similar time course of phenotypic expression and thus shows that continued cell division is not essential to this expression process. In addition, this observation offers methodology that can significantly reduce the investment of time and money for mutation induction determinations in this mammalian cell gene mutation assay.

*Research sponsored jointly by the National Center for Toxicological Research and the Division of Biomedical and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with Union Carbide Corporation.

Conditions Necessary for Quantifying Ethyl Methanesulfonate-Induced Mutations to Purine-Analogue Resistance in Chinese Hamster V79 Cells*

Edward I. Shaw† and Abraham W. Hsie

We have investigated conditions necessary to quantify the relationship between exposure to a mutagen, ethyl methanesulfonate (EMS), and the frequency of mutation induction at the hypoxanthine-guanine phosphoribosyl transferase locus in V79 cells. Maximal expression of potential mutants has been achieved by either subculturing at fewer than 5×10^5 cells per 100-mm dish at 2-day intervals or by daily feeding of cultures. An expression period of 5 days (measured from 1 day after the initiation of treatment with the chemical mutagen) should be allowed, since at least 4 days of expression is required to reach to steady maximum of mutation frequency. It appears that there is no concentration dependence of expression time necessary to reach a plateau of mutation frequency with increasing concentrations of EMS up to 1.6 mg/ml. About 1.25×10^5 cells per 100-mm dish or fewer should be plated for selection to avoid the loss of mutants which occurs at 1.5×10^5 cells per dish, presumably through cross-feeding (metabolic cooperation). The use of 6-thioguanine in hypoxanthine-free medium (supplemented with dialyzed fetal calf serum) appears to be a very stringent condition for selection. Mutation induction by EMS as a function of EMS exposure (EMS concentration × treatment time) increases linearly with concentration up to 12 hr. For these treatment periods, the observed mutation frequencies for EMS are directly proportional to mutagen exposure regardless of the duration of the treatment.

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Utilization of a Quantitative Mammalian Cell Mutation System, CHO/HGPRT, in Experimental Mutagenesis and Genetic Toxicology*

Abraham W. Hsie, David B. Couch,† J. Patrick O'Neill,† Juan R. San Sebastian,†
Patricia A. Brimer, Richard Machanoff, James C. Riddle,‡ Albert P. Li,§
James C. Fuscoe,‖ Nancy Forbes, and Mayphoon H. Hsie

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Quantitative Mammalian Cell Genetic Toxicology: Study of the Cytotoxicity and Mutagenicity of Seventy Individual Environmental Agents Related to Energy Technologies and Three Subfractions of a Crude Synthetic Oil in the CHO/HGPRT System*

Abraham W. Hsie, J. Patrick O'Neill,† Juan R. San Sebastian,† David B. Couch,†‡
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Quantitative Analyses of Radiation- and Chemical-Induced Lethality and Mutagenesis in Chinese Hamster Ovary Cells

Abraham W. Hsie, J. Patrick O'Neill,* David B. Couch,* Juan R. San Sebastian,*
Patricia A. Brimer, Richard Machanoff, James C. Fuscoe,† James C. Riddle,‡
Albert P. Li,§ Nancy L. Forbes, and Mayphoon H. Hsie

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Comparative Mutagenicity of Alkylsulfate and Alkanesulfonate Derivatives in Chinese Hamster Ovary Cells*

David B. Couch,† Nancy L. Forbes, and Abraham W. Hsie

Mutation induction and cell killing produced by selected alkylsulfates and alkanesulfonates have been quantitated using the Chinese hamster ovary/hypoxanthine-guanine phosphoribosyl transferase (CHO/HGPRT) system. Dose-response relationships of cytotoxicity and mutagenicity are presented for two alkysulfates [dimethylsulfate (DMS), diethylsulfate (DES)] and three alkyl alkanesulfonates [methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS), and isopropyl methanesulfonate (iPMS)]. Under the experimental conditions employed, cytotoxicity decreased with the size of the alkyl group. DMS was more toxic than DES, and MMS was more toxic than EMS and iPMS. All agents produced linear dose-response of mutation induction: DMS was more mutagenic than DES, and MMS was more mutagenic than EMS and iPMS based on mutants induced per unit mutagen concentration. However, the following relative mutagenic potency was observed when comparisons were made at 10% survival: DES > DMS; EMS > MMS > iPMS.

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†Postdoctoral investigator, Carcinogenesis Training Grant CA 05296 from the National Cancer Institute.

Mutagenicity and Cytotoxicity of Congeners of Two Classes of Nitroso Compounds in Chinese Hamster Ovary Cells*

David B. Couch† and Abraham W. Hsie

The induction of mutation by certain nitrosamidines and nitrosamides has been quantitated utilizing the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus in Chinese hamster ovary cells. Dose-response relationships for cytotoxicity and mutagenicity are presented for *N*-methyl-*N*-nitrosourea (MNU), *N*-ethyl-*N*-nitrosourea (ENU), *N*-butyl-*N*-nitrosourea (BNU), *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), and *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine (ENNG). Based on the concentration of each agent required to kill 90% of the cells, the following order of cytotoxicity was observed: MMNG > ENNG > MNU > ENU > BNU. This is the same order of potency as observed for mutation induction per unit concentration of mutagen.

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†Postdoctoral investigator, Carcinogenesis Training Grant CA 05296 from the National Cancer Institute.

Relationship Between DNA Alkylation and Specific-Locus Mutation Induction by *N*-Methyl- and *N*-Ethyl-*N*-Nitrosourea in Cultured Chinese Hamster Ovary Cells (CHO/HGPRT System)*

Heinz-Walter Thielmann,† Claus H. Schröder,‡ J. Patrick O'Neill, Patricia A. Brimer, and Abraham W. Hsie

Chinese hamster ovary (CHO) cells in culture were utilized to determine the cytotoxicity, specific-locus mutation induction, and DNA alkylation which result from treatment of the cells with a range of concentrations of *N*-methyl-*N*-nitrosourea (MNU) or *N*-ethyl-*N*-nitrosourea (ENU). With [^{3}H]MNU, methylation of DNA was found to increase linearly over the concentration range 0.43–13.7 m*M* (mean value, 56.7 pmol residue per μmol nucleotide per m*M*). With [1-^{3}H]ENU, ethylation was linear over the concentration range 1.7–26.8 m*M* (mean value, 3.8 pmol residue per μmol nucleotide per m*M*). Mutation induction at the

hypoxanthine-guanine phosphoribosyl transferase locus was quantified by determination of the frequency of resistance to 6-thioguanine under stringently defined selection conditions. The mutation frequency increased linearly with MNU or ENU concentration (0.01–2.0 m*M*); mean values were 2800 and 840 mutants per 10^6 clonable cells per m*M* respectively. At equal levels of DNA alkylation, ENU was found to be approximately 4.5 times as mutagenic as MNU.

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Mutagenicity of Dimethylnitrosamine and Ethyl Methanesulfonate as Determined by the Host-Mediated CHO/HGPRT Assay*

Abraham W. Hsie, Richard Machanoff, David B. Couch,† and J. Michael Holland

Host-mediated assays have been developed to allow determination of the mutagenic potential of promutagens and procarcinogens which require metabolic activation to exert their effects on indicator organisms. We report here the development of the host (mouse)-mediated CHO/HGPRT system using the procarcinogen dimethylnitrosamine (DMN) as a model agent. Using a 2-hr treatment time, we observed a linear dose-response relationship up to 250 mg of DMN per kilogram of body weight. At 100 and 500 mg/kg DMN, mutation induction increased with time up to at least 6 hr. DMN was not mutagenic when tested *in vitro*. Athymic (nude) mice, their phenotypically normal littermates, or BALB/c mice of both sexes were found to be suitable as hosts. A time and dose dependency of induced mutation frequency by a direct-acting agent, ethyl methanesulfonate (EMS), was observed in both the *in vitro* and the host-mediated assays.

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†Postdoctoral investigator, Carcinogenesis Training Grant CA 05296 from the National Cancer Institute.

Mutagenicity of Heterocyclic Nitrogen Mustards (ICR Compounds) in Cultured Mammalian Cells

J. Patrick O'Neill,* James C. Fuscoe,† and Abraham W. Hsie

The mutagenicity of six heterocyclic nitrogen mustards (ICR compounds) has been determined in a cultured mammalian cell system by use of resistance to the purine analog 6-thioguanine to select for mutation induction at the hypoxanthine-guanine phosphoribosyl transferase locus in Chinese hamster ovary cells. The six compounds tested are ICR 191, 170, 292, 372, 191-OH, and 170-OH. The first four contain a single 2-chloroethyl group (nitrogen half-mustard) on the side chain and are mutagenic, with the tertiary amine types (170 and 292) three to five times more mutagenic than the secondary amine types (191 and 372). The remaining two compounds (191-OH and 170-OH) are not mutagenic, indicating that the 2-chloroethyl group is needed for mutation induction.

*Postdoctoral investigator, Carcinogenesis Training Grant CA 05296 from the National Cancer Institute.

†Predoctoral investigator, Grant CA 09104 from the National Cancer Institute.

An Effect of Cell Cycle Position on Ultraviolet Light–Induced Mutagenesis in Chinese Hamster Ovary Cells

James C. Riddle* and Abraham W. Hsie

Using synchronous populations obtained by selectively detaching mitotic cells from cultures grown in monolayer, we demonstrate here that Chinese hamster ovary (CHO) cells exhibit a differential sensitivity to mutation induction by UV as a function of position in the cell cycle. When mutation induction to 6-thioguanine (TG) resistance is monitored, several maxima and minima are displayed during cell cycle traverse, with a major maximum occurring in early S phase. Although cells in S phase are more sensitive to UV-mediated cell lethality than those in G_1 or G_2/M phases, there is not a strict correlation with induced mutation frequency. Fluence-response curves obtained at several times during the cell cycle yield D_q values approximating 6 J/m^2. The primary survival characteristic that varies with cell cycle position is D_0, ranging from 2.5 J/m^2 at 6 hr after mitotic selection to 5.5 J/m^2 at 11 hr afterward. Based on studies with asynchronous, logarithmically growing populations, as well as those mitotically selected to be synchronous, the optimum phenotypic expression time for induced TG resistance is 7–9 days and is essentially independent of both UV fluence and position in the cell cycle. All isolated mutants have altered hypoxanthine-guanine phosphoribosyl transferase (HGPRT) activity, and no difference in the residual level of activity was detected among isolated clones receiving UV radiation during G_1, S, or late S/G_2 phases of the cell cycle. Changes in cellular morphology during cell cycle traverse do not contribute to the differential susceptibility to UV-induced mutagenesis.

*Predoctoral investigator, Grant GM 1974 from the National Institute of General Medical Sciences.

Growth Arrest of Chinese Hamster Ovary Cells in Serum-Free Medium: Changes in Cell Morphology and the Intracellular and Prostaglandin-Induced Levels of Cyclic AMP*

J. Patrick O'Neill and Abraham W. Hsie

When Chinese hamster ovary (CHO) cells are shifted from medium that contains serum into serum-free medium, they complete one cell doubling and arrest in the G_1 phase of the cell cycle. During the first 72 hr of arrest, there is little change in intracellular adenosine 3′:5′-phosphate (cAMP) level, and the cells retain their usual epithelial-like morphology. After 96 hr, the cAMP level doubles, the magnitude of the prostaglandin E_1-induced changes in cAMP increases threefold, and the cells convert from a rounded, epithelial-like shape to an elongated, fibroblast-like form. The fact that these biochemical and cellular transitions are subsequent to the growth arrest shows that the cAMP increase is not the cause of the growth arrest but is consistent with a role for cAMP in the control of cell morphology. In addition, these changes point to the importance of the G_1 phase for initiating cAMP-related events.

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Further Characterization of a Chinese Hamster Ovary Cell Variant with an Altered Morphological Response to db-cAMP

L. S. Borman,* D. C. Swartzendruber,† and A. W. Hsie

When Chinese hamster cells of ovarian origin (CHO cells) are treated with db-cAMP, they change from a compact, epithelial-like cell shape to an elongated, fibroblast-like morphology. Accompanying this response are changes in other cell properties, including a reduction in cell agglutinability with plant lectins, a lowered saturation density of growth, and a change in the cell surface from an irregular, knobby membrane to a smooth, ruffling one. Transmission electron microscopy (TEM) studies show that treated CHO cells increase

their microtubule number and microtubule orientation parallel to the new long axis of the cell. A variant of CHO cells exists which does not elongate after treatment; these variant cells do not increase their microtubule orientation after treatment but do appear to increase their number of microtubules. In this study we have further characterized the variant with respect to the pleiotropic effects of db-cAMP treatment on CHO cells. Phase-contrast microscopy shows that although the variant does not elongate after treatment, it does change its rounded cell shape into a more spread and epithelial-like shape. The cell surface of the variant cells is knobby prior to treatment and remains so afterwards. Like the parental cell, the variant reduces its agglutinability to plant lectins and lowers its saturation density of growth after treatment. A TEM study of cells sectioned at three vertical levels of the cell – bottom, middle, and top with respect to the substratum surface – shows that the treated parental cell increases its microtubule number and orientation only at the middle and bottom levels of the cell. The treated variant increases its microtubule number at all three levels of the cell but does not show increased microtubule orientation at any level. We also observe, from our measurements of microtubule number and length, that the variant contains approximately half the quantity (number X length) of microtubules present in parental cells under both control and treated conditions.

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Effects of Six Prostaglandins on the Cyclic AMP System of WI-38 Human Diploid Fibroblasts

J. Patrick O'Neill,* Albert P. Li,† and Abraham W. Hsie

Incubation of VI-38 cells with prostaglandins induces a rapid increase in intracellular levels of adenosine 3′:5′-phosphate (cAMP). At saturating concentrations, prostaglandin E_1 is the most active, followed by (in order of potency) E_2, A_1, A_2, B_1, and $F_{2\alpha}$. Studies of the desensitization of the prostaglandin-induced changes in cAMP level suggest that these cells only have one type of prostaglandin receptor. The effects of this increase in intracellular cAMP level on the cAMP phosphodiesterase and the cAMP-dependent protein kinase activities were studied. In cells incubated with 50-μM prostaglandin E_1 for up to 120 min there was little change in any of the three phosphodiesterase activities. However, the cAMP-dependent protein kinase activity was completely activated in cultures incubated for only 1 min and remained partially activated for 2–4 hr. The degree of activation of the protein kinase paralleled the magnitude of the increase in intracellular cAMP level.

*Postdoctoral investigator, Research in Aging Training Grant HD-00296 from the National Institute of Child Health and Human Development and Carcinogenesis Training Grant CA 05296 from the National Cancer Institute.

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Characterization of Two Adenosine 3′:5′-phosphate-Dependent Protein Kinase Species from Chinese Hamster Ovary Cells

Albert P. Li* and Abraham W. Hsie

Chinese hamster ovary (CHO) cells exhibit several characteristic morphological and physiological responses upon treatment with agents which increase the intracellular level of adenosine 3′:5′-phosphate (cAMP). To better understand the mechanism of these cAMP-mediated responses, we separated two cAMP-dependent protein kinases (protein kinase I and protein kinase II) from the cytosol of CHO cells by DEAE-cellulose chromatography and studied their properties. Protein kinase I is eluted at lower salt concentration than protein kinase II and is stimulable to tenfold of its basal catalytic activity, while protein kinase II is stimulable to only twofold. Both kinases are completely dissociated by cAMP and inhibited by specific cAMP-dependent protein kinase inhibitor. They have similar K_m values for magnesium (~1 mM), cAMP (~60 nM), and ATP (~0.1 mM), and the dissociation constant (K_{dis}) for cAMP (~13 nM) is the same for

both enzymes. However, they appear to have different substrate preferences and cAMP-binding properties in that cAMP bound to protein kinase II exchanges readily with free cAMP, while that bound to protein kinase I is not exchangeable. The native enzymes have different sedimentation coefficients (6.4 S for protein kinase I and 4.8 S for protein kinase II), whereas those of the activated enzymes are the same (2.9–3.0 S). It appears that the two cAMP-dependent protein kinases which differ from each other in their regulatory subunits may play different roles in the mediation of cAMP action in CHO cells.

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Steady-State Kinetics Studies on the Adenosine 3′:5′-phosphate-Dependent Protein Kinase in Chinese Hamster Ovary Cells

Albert P. Li,* David G. Gosslee,† Douglas S. Robson,†‡ and Abraham W. Hsie

Theories of steady-state enzyme kinetics were used to investigate the mechanism of action of the partially purified cyclic AMP-dependent protein kinase from Chinese hamster ovary cells. Evidence is provided that the mechanism is sequential, with the suggestion that it is rapid-equilibrium random sequential. We constructed a statistical procedure to distinguish among ping-pong, ordered-sequential, and rapid-equilibrium random sequential mechanisms. This statistical procedure may be applicable in studying the molecular mechanisms of action of other enzyme systems.

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Repair of DNA Damaged by Mutagenic Metabolites of Benzo[*a*]pyrene in Human Cells*

James D. Regan, A. A. Francis, W. C. Dunn, O. Hernandez,† H. Yagi,‡ and Donald M. Jerina†

The repair of human DNA after damage by known and potential metabolites of benzo[*a*]pyrene has been examined utilizing the bromodeoxyuridine photolysis assay. Repair was characterized as either ultraviolet ("long") or ionizing radiation-type ("short") repair utilizing normal cells and cells deficient in ultraviolet-type repair endonuclease from a patient with xeroderma pigmentosum (XP). We have found that only (±)-7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BP diol epoxide 1) and its diastereomer, (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BP diol epoxide 2) elicit damage to DNA, which is recognizable by the ultraviolet excision repair system in normal human cells. Benzo[*a*]pyrene 4,5-, 9,10-, 11,12-oxides do not elicit damage that is repairable by this repair system. The 1,2-diol-3,4-epoxides from naphthalene have no measurable activity in our assay. These results indicate that both the benzo[*a*]pyrene ring structure and the diol epoxide groups are important in causing the damage to DNA which is repairable by the ultraviolet excision repair system. These results parallel the reported high mutagenic activity of these compounds and support the concept that benzo[*a*]pyrene 7,8-diol-9,10-epoxides may be the ultimate, metabolically activated forms of benzo[*a*]pyrene.

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DNA Repair in Human Fibroblasts Following Exposure to Carcinogens: Absence of Additivity from Combined Treatments

Alex J. Brown,* Ted H. Fickel,†‡ James E. Cleaver,‡ and Raymond Waters

DNA excision repair has been measured in cultured human fibroblasts following single or dual treatments with ultraviolet irradiation, 4-nitroquinoline 1-oxide, or *N*-acetoxy-2-acetylaminofluorene. Two different approaches were used to monitor this repair: the first measured unscheduled DNA synthesis by autoradiography, and the second measured the incorporation of a density-labeled DNA precursor into repaired regions. Both assays indicated that when a single repair-saturating dose of one of the three carcinogens was administered, little stimulation of this level could be observed by additional treatment with one of the other carcinogens. In no instance was there a total additivity of the repair observed by separate saturating doses of these different carcinogens. The data indicate that the repair of lesions induced by these substances may have a common rate-limiting step.

*Predoctoral investigator, Grant GM 1974 from the National Institute of General Medical Sciences.

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Inhibition of DNA Excision Repair in Human Cells by Arabinofuranosyl Cytosine: Effect on Normal and Xeroderma Pigmentosum Cells*

William C. Dunn and James D. Regan

The antineoplastic agent arabinofuranosyl cytosine (ara-C) produces an inhibition of the pyrimidine dimer excision system of human DNA repair. Alkaline sucrose gradient analysis of DNA from normal human skin fibroblasts exposed to 20 J/m^2 of ultraviolet radiation (254 nm) shows an accumulation of DNA single-strand breaks when DNA repair is attempted in the presence of 10-μM ara-C. Cells from complementation groups of xeroderma pigmentosum that are defective in the initial endonuclease incision step of dimer excision show reduced numbers of DNA strand breaks/10^8 daltons when compared with normal cells. Cesium chloride gradient analysis of radioactive precurser uptake during repair replication indicates that ara-C causes a 6–56% reduction in the number of nucleotide bases inserted into the DNA at concentrations of 1 and 10 μM respectively. These concentrations result in the substitution for deoxycytidine (dCyd) by ara-C of 40 and 100%, respectively, in repaired regions. Repair inhibition is reversed by 50% upon removal of ara-C and by >95% with the addition of 100-μM dCyd. Chromatography of digested DNA shows that incorporated ara-C is not removed during dCyd reversal, suggesting that ara-C incorporation *per se* does not play a significant role in the inhibition of repair synthesis. The repair inhibition observed here is dependent on 2-mM hydroxyurea, presumably due to reduction in the intracellular pool of dCyd. The overall results suggest that at noncytotoxic doses ara-C is a weak competitive inhibitor of DNA polymerases associated with ultraviolet-induced excision repair.

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Inhibition of DNA Repair in Ultraviolet-Irradiated Human Cells by Hydroxyurea*

Andrew A. Francis, R. Dean Blevins,† William L. Carrier, David P. Smith, and James D. Regan

The effect on DNA repair in UV-irradiated human skin fibroblasts by hydroxyurea (HU) has been examined in this study using three independent methods for measuring DNA repair: the 5-bromodeoxyuridine photolysis assay which measures DNA repair replication, chromatographic measurement of thymine-containing dimers, and measurement of specific UV-endonuclease-sensitive sites in irradiated DNA. Little effect of HU

was observed at the concentration of 2 m*M*, which is often used to inhibit semiconservative DNA synthesis; however, 10-m*M* HU resulted in marked inhibition (65–70%) of excision repair. This inhibition was accompanied by a possible doubling in the size of the repaired region. The accumulation of large numbers of single-strand breaks following UV irradiation and HU incubation seen by other investigators was not observed with the normal skin fibroblasts used in this study. A comparison of HU effects on the different DNA repair assays indicates inhibition of one step in DNA repair also results in varying degrees of inhibition of other steps as well.

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Increased Amounts of Hybrid (Heavy/Heavy) DNA in Bloom's Syndrome Fibroblasts*

Raymond Waters, James D. Regan, and James German†

The nuclear DNA of fibroblasts from patients suffering with Bloom's syndrome, density labeled for less than one round of DNA replication to give heavy/light molecules, was examined for spontaneous amounts of heavy/heavy DNA (hybrid DNA). When compared to normal fibroblasts the Bloom's syndrome cells exhibited a sixfold increase in such DNA.

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Serine Requirement of Bone Marrow Cells of Experimental Animals*

F. M. Faulcon, James B. Jones, and James D. Regan

In an effort to find animal models for serine antimetabolite studies, we examined the serine requirement of bone marrow cells of the dog, cat, rabbit, guinea pig, rat, and mouse. From measurements of radioactive precursor uptake, we found that extrinsic serine is required for optimum *in vitro* macromolecular synthesis by dog and rabbit marrow cells. Marrow cells from the other animals did not require extrinsic serine. It appears that the dog and rabbit would make suitable *in vivo* test systems for serine antimetabolite studies.

*Research sponsored jointly by the National Cancer Institute under Interagency Agreement 40-5-63 and the Division of Biomedical and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with Union Carbide Corporation.

The Removal of UV-Induced Pyrimidine Dimers from the Replicated and Unreplicated DNA of Human Fibroblasts

Raymond Waters

Excision repair in UV-irradiated human fibroblasts has been examined in portions of DNA replicating after irradiation versus those remaining unreplicated. Two approaches – one using a UV-endonuclease to estimate pyrimidine dimers remaining in DNA, the other using density labeling to measure excision resynthesis – indicate that the extent of repair is the same for both replicated and unreplicated DNA.

Repair of DNA in Replicated and Unreplicated Portions of the Human Genome

Raymond Waters

Portions of the human genome that have replicated after UV irradiation and those that remain unreplicated have both been examined for the distribution of pyrimidine dimers and the extent of repair replication following their removal. The data indicate that the number of unrepaired dimers and the extent of repair replication seen after their excision are equal in the replicated and unreplicated DNA. Furthermore, the daughter strand of replicated DNA is larger than the average interdimer distance found in the parental strand. Hence, DNA replication is clearly capable of getting past pyrimidine dimers, and a preferential repair of such lesions in DNA that is about to be or has been replicated does not operate to any visible extent in human fibroblasts.

The Production of Chromosome Aberrations in Various Mammalian Cells by Triethylenemelamine*

H. E. Luippold, P. C. Gooch, and J. G. Brewen

The cytogenetic effects of triethylenemelamine (TEM) were studied using five different mammalian tissues. Treatments of 0.1 and 0.2 mg/kg TEM on differentiating mouse spermatogonia and bone marrow cells showed no significant differences in the frequency of chromosomal aberrations produced in these two tissues. At higher doses, however, the sensitivities of the two tissues appear to be different. The frequency of aberrations varies with time after treatment, with the greatest amount occurring at the later fixation times. Results of an experiment on primary spermatocytes indicated a correlation between the frequency of chromosome aberrations and DNA replication. Human peripheral leukocytes were utilized in an attempt to clarify the cell stage specificity of TEM-induced chromosome aberrations. Cultures were treated with TEM prior to PHA stimulation (G_0) as well as various time intervals after stimulation (late G_1, S, and G_2). The most sensitive stages of the cell cycle to aberration induction were late G_1 and S, with chromatid aberrations the predominate type. A very low yield of chromosome-type damage was observed with the G_0- and G_1-treated stages. The experiments described tend to support the idea that TEM is most effective at inducing aberrations when an intervening round of DNA replication has occurred.

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A Mechanism for the Induction of Chromosome Aberrations in Human Lymphocytes by X Rays

R. Julian Preston

Abstract not available.

X-Ray Stage Sensitivity of Mouse Oocytes and Its Bearing on Dose-Response Curves

J. G. Brewen and H. S. Payne

A detailed cytogenetic study of maturing mouse oocyte radiosensitivity was performed. Oocytes were collected at various intervals ranging from 1½ days to 28½ days after irradiation with 50, 100, 200, and 300 R of acute X rays. It was observed that the sensitivity to chromatid aberration induction varied greatly over this time span. Sensitivity was lowest at the shortest interval before ovulation and gradually increased up to day 9½, from whence it remained constant until insufficient numbers of oocytes could be collected. The data were analyzed in three ways. First, the data from all time intervals at each dose were pooled;

second, the data from the least-sensitive time intervals at each dose were pooled; and third, the data from the period of uniform sensitivity at each dose were pooled. Dose-response regression analyses were done on these pooled data, and the best fits obtained were to the models $Y = a + bD + cD^2$ and $Y = a + cD^2$ for both deletions and interchanges. This result is interpreted as meaning the aberrations result from a predominantly two-track process. The cytogenetic data were compared to specific-locus mutation induction in comparable oocyte stages, and an excellent qualitative similarity in dose-response characteristics was observed. This similarity is interpreted to mean both events are induced by the same mechanism and that the large-dose-rate effect observed for both events is a reflection of the two-track component in the dose-response curves.

Assessing Chemical Mutagens: The Risk to Humans

J. Grant Brewen

Abstract not available.

Comparative Mutagenesis: Mammalian *In Vitro* Cytogenetics

J. G. Brewen, A. T. Natarajan,* and G. Obe†

Abstract not available.

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Embryonic Hemoglobins in α-Thalassemic Mice*

Raymond A. Popp, Blaine S. Bradshaw, and Gerald P. Hirsch

Embryonic hemoglobins in heterozygous α-thalassemic and normal fetuses were compared to study the effects of the deficient α chain on the synthesis of hemoglobins in the nucleated embryonic erythrocytes derived from the fetal yolk sac. Visual inspection of embryonic hemoglobins following acrylamide gel electrophoresis suggested that less hemoglobin EII ($\alpha_2 y_2$) was formed in α-thalassemic heterozygotes between 12½ and 14½ days of gestation. Quantitation of *in vitro* synthesis between 11½ and 13½ days of gestation confirmed that EII was synthesized less rapidly in α-thalassemic fetuses. In contrast, the synthesis of EIII ($\alpha_2 z_2$) was higher in α-thalassemic than in normal fetuses at 12½ and 13½ days of gestation. Measurements of the synthesis of individual chains in EI ($x_2 y_2$) and EII showed that x-chain synthesis was normal and that α-chain synthesis was deficient in α-thalassemic fetuses at 11½ and 12½ days of gestation. There is still no proof for close linkage of x- and α-chain genes in chromosome 11. Differences in the electrophoretic patterns of embryonic hemoglobins of α-thalassemic and normal fetuses can be explained by normal synthesis of x chains, deficient synthesis of α chains, and a higher affinity of z than y for the reduced amount of α chain present in the nucleated embryonic erythrocytes of α-thalassemic mice.

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Amino Acid Sequence of a Progesterone-Binding Protein*

Raymond A. Popp, Kerry R. Foresman,† L. David Wise,† and Joseph C. Daniel, Jr.†

The amino acid sequence of blastokinin, also called uteroglobin, has been determined by a combined study of both the intact, native molecule and the peptide fragments resulting from tryptic and chymotryptic

digestion. Sequence analyses performed by automated methods and by sequential digestion employing leucine aminopeptidase and carboxypeptidase Y demonstrate that blastokinin is a dimer of identical 69 amino acid subunits held together in parallel orientation by two disulfide bridges at positions 3 and 68. The polypeptide chains are further characterized by the absence of tryptophan residues and by single residues of histidine and tyrosine at positions 8 and 21 respectively. Six of eight amino acids, positions 17 to 24, near the progesterone binding site of blastokinin contain a hydroxyl group. Knowledge of the chemistry of this receptor site, the first to be elucidated, should allow better perspectives of the chemistry of molecules in normal tissues dependent on progesterone for growth and development, as well as compounds that could act as cancer antagonists for endocrine therapy of hormone-dependent tumors.

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Erroneous Incorporation of Cysteine into a Rat Seminal Vesicle Protein: Effect with Age and X Rays

Blaine S. Bradshaw,* Raymond A. Popp, and Gerald P. Hirsch

A cysteine-minus protein from rat seminal vesicle gland was isolated and used to measure the average substitution frequency of cysteine for other genetically coded amino acids. Nine Fischer 344 (CDF/Crl Br) male rats, ranging from 4 to 25 months of age, were used as the source of seminal fluid. Two of these rats were used as positive controls and were given two doses of X rays of 150 R each. Proteins of the seminal vesicles of the rats were labeled in situ with L-[^{3}H]proline and L-[^{35}S]cysteine. The specific activity of [^{3}H]proline and [^{35}S]cysteine in the newly synthesized protein was used to establish the average substitution frequency of cysteine for other genetically coded amino acids in the cysteine-minus protein. The average substitution frequencies ranged from 1.13×10^{-5} to 4.53×10^{-5} for normal aging rats; for the two irradiated rats it was 4.59×10^{-5} and 9.68×10^{-5}. We describe a system in which the product (a protein-lacking genetically coded cysteine) of a long-lived cell can be used as an indicator of mutagenic and carcinogenic effects.

*Postdoctoral investigator, Grant AG 00028 from the National Institute on Aging.

Spontaneous Mutations Balance Reproductive Selective Advantage and Genetically Determine Longevity

Gerald P. Hirsch

The theory is presented that the genetic potential for longevity of a given species in a protected environment is determined by the rate of predation in the wild and by the frequency of spontaneous mutations. A common genetically determined life span representative of rare, long-lived individuals in the wild results from the slight selective advantage conferred by genotypes which do not cause early death. The selective advantage of reproductive rare survivors is limited by the rate of spontaneous mutations from a common potential longevity to those which limit life span to shorter times. The equilibrium frequency of life-limiting genes increases exponentially with increasing age when the rate of mutations is uniform for different degrees of life shortening.

Life-Span Studies of Erythrocytes Taken from Old and Young Mice

M. W. Francis, R. A. Popp, L. H. Smith, and G. P. Hirsch

As mice age, the hematocrit value, mean corpuscular volume, and mean corpuscular hemoglobin concentration have been shown to decline steadily. Mouse erythrocytes also show a steady decline in mean corpuscular volume as they age *in vivo*. These observations suggest that the physical differences between erythrocytes circulating in young and old mice could reflect an increase in the average age of the erythrocyte population in old animals. Accordingly, young (4 month old) and old (24–29 month old) C3H mice received an injection of ^{51}Cr-labeled erythrocytes from young or old C3H mice, and the clearance rate of the ^{51}Cr-labeled cells was determined for 28 days. Erythrocytes from both the old and young donors were cleared at the same rate in both young and old recipients; half-clearance time was approximately 13 days.

This suggests that the mean age of circulating erythrocytes in young or old C3H mice does not differ and that erythrocyte age does not explain the host age-associated reduction of hematocrits and mean corpuscular volumes in old mice.

Hemopoietic Stem Cell Heterogeneity: Use of Cell Cycle–Specific Drugs to Look for Age-Associated Alterations

Diana M. Popp and Raymond A. Popp

Hemopoietic tissue is vulnerable to perturbations, and data show that it is an appropriate tissue in which to look for age-associated alterations. This tissue has a high regenerative capacity, is composed of a heterogeneous population of stem cells that are capable of self renewal or differentiation, or both, and is sustained by a pool of resting cells. The heterogeneity of bone marrow has made characterization of the cellular elements difficult. Techniques commonly used to identify and quantify the various maturation levels of hemopoietic stem cells and the limitations of these techniques are discussed. Most techniques used to assay age-associated changes in bone marrow have not differentiated between specific cellular alterations or shifts in the distribution of the cellular elements. In particular, it has been difficult to determine the stability of the nondividing stem cell because of the low incidence of this cell (6/1000) and the lack of a specific assay for this important cell type. The use of cell cycle–specific drugs has provided quantitative information on specific subpopulations of hemopoietic stem cells and seems to be the most promising approach toward determining qualitative and quantitative differences in the hemopoietic stem cells of young and old individuals.

Age-Associated Changes in T-B Cell Cooperation Demonstrated by the Allogeneic Effect

Diana M. Popp and Mary Francis

The allogeneic effect results in an increase in the number of antibody-producing cells (PFC) when the allogeneically stimulated lymphoid cells and the antibody-producing cells are from young individuals. This increase depends on the interaction of allogeneically stimulated T-cells and precursor plasma cells (B-cells). Age-associated deficiencies in these two cell populations were studied in these experiments by use of allogeneically stimulated T-cells from old donors or responding B-cells from old recipients.

These experiments showed that lymphocytes from 20-month-old mice were capable of inducing an increase in PFC in young recipients. However, the allogeneic effect was ineffectual in 12-month-old mice. These data suggest that the T-helper function is equally capable in mice 4 to 20 months old but that the B-cell is not as effectively recruited. The possible causes for limited B-cell recruitment are discussed.

A Comparison of Heat and Interchromosomal Effects on Recombination and Interference in *Drosophila melanogaster*

R. F. Grell

Heat and interchromosomal effects on recombination have been compared for 23 regions comprising the predominantly euchromatic portions of the five arms of the *Drosophila* genome. Patterns of response are strikingly similar, with both modifiers causing proximal and distal increases and minimal effects in the middle of the arms. Changes in interference for the same regions in the presence of the two modifiers reveal little similarity, except for the X chromosome. The question of independent control of interference and recombination, as well as alternatives for their temporal sequence, is discussed. Recombination response to the two modifiers in the centric heterochromatin of chromosome 2 is markedly different from that found in euchromatin. The interchromosomal effect is absent here, whereas heat induces an increase roughly an order of magnitude greater than that found in euchromatin and totally unlike the lack of response in the proximal heterochromatin of the X chromosome. It is proposed that the sequestering of DNA satellite I (thermal dissociation 9–20° lower than that of the other major satellites) in the centromeric heterochromatin of chromosome 2 (but not in X or 3) may account for the increase.

Time of Recombination in the *Drosophila melanogaster* Oocyte: Evidence from a Temperature-Sensitive Recombination-Deficient Mutant

R. F. Grell

A temperature-sensitive recombination-deficient mutant, *rec-1*^{26}, has been isolated which permits high frequencies of recombination at the permissive temperature (25°C) but greatly reduces recombination at the restrictive temperature (31°C). The sensitive period for response of female germ cells carrying this mutant to the restrictive temperature has been defined. Sensitivity begins very close to the time the oocyte enters premeiotic interphase and initiates DNA synthesis; it continues for the duration of premeiotic-S, and it terminates with the completion of S. This time span precisely coincides with the sensitive period for enhancement of recombination by heat in the normal genome and is further characterized by the presence of the synaptonemal complex. These results provide compelling evidence for identifying premeiotic-S as the time of meiotic recombination.

High-Frequency Recombination in Centromeric and Histone Regions of *Drosophila* Genomes

R. F. Grell

Previous studies have shown that the sensitive period for heat enhancement of recombination throughout the *Drosophila* genome and heat induction of recombination in chromosome 4 coincides with premeiotic-S. DNA synthesis in eukaryotes has been shown to be asychronous with heterochromatin replicating later than euchromatin. Heat treatment of *Drosophila* oocytes at sequential intervals during premeiotic-S elicits increases in recombination that follow a well-ordered pattern. To investigate the possibility that the pattern of heat response parallels that of replication, the centromeric heterochromatin of chromosome 2 and the contiguous histone region have been examined for the time of their recombinational response to heat during the S phase. We find that late replication in the centromeric heterochromatin of chromosome 2 is correlated with a dramatic increase in recombination induced by heat treatment during the last two-fifths of the S period. Response in the histone region occurs still later, during the last fifth of the S phase. A level of response roughly two orders of magnitude greater than the average encountered in the genome has been found in these regions. A close temporal correlation between replication and recombination in specific regions is suggested.

Developmental Changes of Sepiapterin Synthase Activity Associated with a Variegated Purple Gene in *Drosophila melanogaster*

J. E. Tobler,* J. J. Yim,† E. H. Grell, and K. B. Jacobson

A variegated position effect on the autonomous gene purple has been studied enzymologically in *Drosophila melanogaster*. Sepiapterin synthase, the enzyme associated with pr^+, was examined for activity in different developmental stages of the fly. The results indicate that T(Y:2)pr^{c5};*cn*/pr^{c4},*cn* flies (flies in which pr^+ has been translocated and which exhibit variegation) have a reduced amount of enzyme activity as compared with both Oregon-R and pr^1 flies. This reduction in activity was not found in larval stages, which suggests that the inactivation process probably occurs in late larval or early pupal stages. These results and their biological implications are discussed.

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†Postdoctoral investigator, subcontract 3322 from the Biology Division of Oak Ridge National Laboratory to the University of Tennessee.

Gap-Filling Repair Synthesis Induced by Ultraviolet Light in a *uvr*⁻ Mutant of *Bacillus subtilis**

Charles T. Hadden

DNA repair synthesis was studied in one wild-type and two mutant strains of *Bacillus subtilis* that are defective in excision of pyrimidine dimers. The cells were irradiated with ultraviolet light, and 6-(*p*-hydroxyphenylazo)-uracil was used to block replicative synthesis, allowing only repair synthesis. One of the mutants (*uvs-42*) results in a severe inhibition of incision, dimer excision, and repair synthesis. In contrast, the other mutant (*uvr-1*) slowly incises and excises dimers and does repair synthesis in patches which appear to be several-fold longer than in the wild-type strain, apparently because large gaps are produced at excision sites. The results indicate that the primary defect in *uvs-42* cells is in initiation of dimer excision, whereas the *uvr-1* mutation appears to be a defect in the exonuclease normally used to complete dimer excision.

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Pyrimidine Dimer Excision in a *uvr*⁻ Mutant of *Bacillus subtilis**

Charles T. Hadden

A technique that allows the measurement of small numbers of pyrimidine dimers in the DNA of cells of *Bacillus subtilis* irradiated with ultraviolet light has been used to show that a strain mutant at the *uvr-1* locus is able to excise pyrimidine dimers. Excision-repair in this strain is slow, but incision may not be rate limiting because single-strand breaks in DNA accumulate under some conditions. Excision-repair probably accounts for a liquid-holding recovery previously reported to occur in this strain. Recombinational exchange of pyrimidine dimers into newly replicated DNA is readily detected in *uvr-1* cells, but this type of repair does not account for more than a minor fraction of the dimers removed from parental DNA. Excision-repair in the *uvr-1* strain is inhibited by a drug which complexes DNA polymerase III with gapped DNA. This inhibition may be limited to a number of sites equal to the number of DNA polymerase III molecules, and it is inferred that large gaps are produced by excision of dimers. It is concluded that the *uvr-1* gene product may be an exonuclease that is essential for efficient dimer excision.

*Research sponsored jointly by U.S. Department of Energy contract EY-76-S-05-4568 and the Division of Biomedical and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with Union Carbide Corporation.

Measurement of Pyrimidine Dimers in Spheroplasts of *Bacillus subtilis**

C. T. Hadden

A method is described for making spheroplasts of *Bacillus subtilis* which are permeable to exogenous enzymes. Conditions are described for measuring small numbers of pyrimidine dimers in the DNA of UV-irradiated cells by use of a partially purified *Micrococcus luteus* extract containing an enzyme specific for pyrimidine dimers. The system will detect as few as 10–12 pyrimidine dimers per genome.

*Research sponsored jointly by Energy Research and Development Administration contract AT(40-1)4568 and the Division of Biomedical and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with Union Carbide Corporation.

Quantitation of Pyrimidine Dimers in *Bacillus subtilis*

Charles T. Hadden

Abstract not available.

Differential Sensitivity of DNA Replication and Repair in Permeabilized *Escherichia coli* Exposed to Various Monofunctional Methylating or Ethylating Agents*

G. R. Hellermann and Daniel Billen

Escherichia coli cells made permeable to deoxynucleoside triphosphates by brief treatment with toluene (permeabilized) were used to measure the effect of the following chemical alkylating agents on either DNA replication or DNA repair synthesis: MMS, EMS, MNU, ENU, MNNG, and ENNG. Replication of DNA in this psuedo-*in vivo* system was completely inhibited 10–15 min after exposure to MMS at concentrations of 5 m*M* or higher or to MNU or MNNG at concentrations of 1 m*M* or higher. The ethyl derivatives of the alkylating agents were less inhibitory than their corresponding methyl derivatives, and inhibition of DNA replication occurred in the following order: EMS < ENNG < ENU. Maximum inhibition of DNA replication by all of the alkylating agents tested except EMS occurred at a concentration of 20 m*M* or lower. The extent of replication in cells exposed to EMS continued to decrease with concentrations of EMS up to 100 m*M* (the highest concentration tested).

The experiments in which the inhibition of DNA replication by MMS, MNU, or MNNG was measured were repeated under similar assay conditions except that a density label was included and the DNA was banded in CsCl gradients. The bulk of the newly synthesized DNA from the untreated cells was found to be of the replicative (semiconservative) type. The amount of replicative DNA decreased with increasing concentration of methylating agent in a manner similar to that observed in the incorporation experiments.

Polymerase I (Pol I)-directed DNA repair synthesis induced by X-irradiation of permeabilized cells was assayed under conditions that blocked the activity of DNA polymerases II and III. Exposure of cells to MNNG or ENNG at a concentration of 20 m*M* resulted in reductions in Pol I activity of 40 and 30%, respectively, compared with untreated controls. ENU was slightly inhibitory to Pol I activity, whereas MMS, EMS, and MNU all caused some enhancement of Pol I activity.

These data show that DNA replication in a pseudo-*in vivo* bacterial system is particularly sensitive to the actions of known chemical mutagens, whereas DNA repair carried out by the Pol I repair enzyme is much less sensitive and in some cases apparently unaffected by such treatment. Possible mechanisms for this differential effect on DNA metabolism and its correlation with current theories of chemically induced mutagenesis and carcinogenesis are discussed.

*Research sponsored jointly by the U.S. Department of Energy under contract EY-76-S-05-4568 and the Division of Biomedical and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with Union Carbide Corporation.

An Approach to Setting Radiation Standards*

H. I. Adler and A. M. Weinberg†

Abstract not available.

*Research supported by the U.S. Department of Energy under contract with Union Carbide Corporation and Oak Ridge Associated Universities.

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The Relation of Repair Phenomena to Mutation Induction in Bacteria

R. F. Kimball

The relation of various repair processes to mutation induction by radiation and chemicals is discussed for various species of bacteria. A variety of repair processes have been identified at the molecular level that can eliminate many kinds of potentially mutagenic lesions before they can be converted to final mutation. Fixation often, but not always, occurs at replication. A number of mutagens, including ultraviolet light, ionizing radiation, and a number of chemicals, induce an error-prone process, perhaps a modification of the proofreading system, that allows bacteria to survive after potentially lethal damage at the expense of making errors. Some mutagens, notably monofunctional alkylating agents and base analogues, produce mutations by other processes. Even in these cases, repair processes play an important role. There is some evidence that error-free as well as error-prone repair processes can be induced. A brief discussion is given of the relation of these findings to the practical problems of hazards estimations.

The Role of Prereplication and Postreplication Processes in Mutation Induction in *Haemophilus influenzae* by *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine

R. F. Kimball, S. W. Perdue, and M. E. Boling

Studies were carried out on the repair and fixation of premutational damage induced in *Haemophilus influenzae* by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG). The studies employed a temperature-sensitive DNA elongation mutant (*dna9*) and its combinations with mutants defective in pyrimidine dimer excision (*uvr1*, *uvr2*) and in recombination (*rec1*). The *dna9* mutant is shown to be leaky, allowing about 1% the normal rate of DNA synthesis at the restrictive temperature. Repair of premutational lesions was detected by a decline in mutation frequency with increasing delay in DNA replication in *dna9* at the restrictive temperature. This repair is unaffected by the pyrimidine dimer excision system. Mutation fixation was detected by the ability of DNA from treated and then lysed cells to transfer mutants to recipient cells by transformation. Some fixation occurred at the restrictive temperature but much less than at the nonrestrictive temperature, suggesting that an appreciable minority of the mutations resulted from lesions introduced near the replication fork but that the majority of mutations arise from lesions introduced at some distance from the fork, perhaps randomly. The DNA synthesized immediately after MNNG treatment is of lower molecular weight than normal and returns to normal with time. This return is blocked in the *recl* mutant, suggesting that recombination is involved. The possible role of this process in MNNG mutagenesis is discussed.

Development of a Culture Medium for Growing *Xenopus laevis* Oocytes

Robin A. Wallace, Ziva Misulovin, Donald W. Jared, and H. Steven Wiley*

A defined medium for growing defolliculated *Xenopus laevis* oocytes was developed by monitoring vitellogenin incorporation. Optimum conditions were achieved by use of 50% Liebovitz L-15 medium supplemented with 1 m*M* L-glutamine, 15 m*M* Hepes, 5 mg/ml vitellogenin, and 1 μg/ml insulin (final pH = 7.8).

Stage IV/V oocytes remained morphologically normal in this medium for at least 2 weeks and grew at an average rate of 0.25 $mm^3 \cdot week^{-1}$. This is the first time defolliculated oocytes from any vertebrate have been grown *in vitro*.

*Predoctoral investigator, Grant T32 GM 07431 from the National Institute of General Medical Sciences.

Long-Term Growth and Differentiation of *Xenopus* Oocytes in a Defined Medium

Robin A. Wallace and Ziva Misulovin

Xenopus laevis oocytes over a size range of 0.15–0.78 mm^3 were dissected from their follicles and cultured in a defined medium for up to 28 days. Oocytes grew at average rates of 0.021 $mm^3 \cdot day^{-1}$ in the absence of insulin and 0.030 $mm^3 \cdot day^{-1}$ in the presence of insulin. The latter average growth rate corresponds to the fastest growth rate reported to date for oocytes *in vivo*. Oocytes grown *in vitro* can reach a size of at least 1.43 mm^3, which is larger than the maximum size generally found *in vivo*. During growth *in vitro*, oocytes also acquire both a normal pigment pattern and, once they reach about 0.7 mm^3, the ability to undergo complete maturation as a response to externally applied progesterone. These results represent the first time that vertebrate oocytes freed of their follicular investments have been shown to grow and differentiate *in vitro*.

Preliminary Studies on the Turnover of Endogenous, Microinjected, and Sequestered Protein in *Xenopus* Oocytes

Robin A. Wallace and Thomas G. Hollinger*

Pulse-labeled oocyte proteins were found to have a maximum average half-life of 73 hr. In general, larger peptides underwent degradation at a faster rate than smaller peptides. In this respect, oocytes are similar to most other cells. Microinjected ^{125}I-labeled bovine serum albumin (BSA) was degraded over a 40-hr period with a half-life of 20–30 hr, regardless of the method of protein labeling, culture medium employed, size of oocyte microinjected, or hormonal history of the oocyte. The last two results, if applicable to oocyte proteins in general, imply that protein catabolism is constant throughout the later stages of oogenesis and that growth is primarily regulated by a stimulation of anabolism. Individual proteins microinjected into oocytes undergo rates of degradation consistent with turnover rates obtained in other systems. Sequestered ^{125}I-labeled BSA is only partially (40%) degraded, which indicates that, unlike microinjected ^{125}I-labeled BSA, it has access to a cytoplasmic compartment (yolk platelets?) within which it is relatively stable.

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The Origin of Yolk-DNA in *Xenopus laevis*

Lee Opresko,* H. Steven Wiley,† and Robin A. Wallace

Xenopus laevis serum and plasma was found to contain an average of 25 μg DNA/ml. Isolated *X. laevis* oocytes incubated in medium containing 25 μg DNA/ml labeled with either ^{125}I, ^{32}P, or ^{14}C and from three different sources (bovine, *Escherichia coli* and *X. laevis*), incorporated the label at an average rate of 0.11 $ng \cdot mm^{-2} \cdot hr^{-1}$. Sucrose gradient fractionation of oocytes revealed that 40–75% of the acid-precipitable label incorporated was associated with the yolk platelets. Additional incubations of oocytes in unlabeled medium demonstrated that the DNA incorporated into the yolk platelets was undergoing turnover; only 20% of the yolk-associated DNA was still present after a 1-week incubation. Our data suggest that yolk-DNA arises by the adventitious uptake of DNA present in the maternal serum by vitellogenic oocytes.

*Postdoctoral investigator, subcontract No. 3322 from the Biology Division of Oak Ridge National Laboratory to the University of Tennessee.

†Predoctoral investigator, Grant GM 07431 from the National Institute of General Medical Sciences.

Three Different Molecular Weight Forms of the Vitellogenin Peptide from *Xenopus laevis**

H. Steven Wiley and R. A. Wallace

Vitellogenin derived from the blood of estrogen-treated *Xenopus laevis* is comprised of at least three different polypeptides, designated α-, β-, and γ-vitellogenin. The molecular weights of the three polypeptides are 197,000, 188,000, and 182,000, respectively, and the ratio of their relative amounts is approximately 2α:2β:1γ.

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An Autoradiographic Study of Vitellogenesis in the Squid, *Loligo pealei**

Kelly Selman† and Robin A. Wallace

The structure of the vitellogenic (Stage IV) ovarian follicle in *Loligo pealei* is described. Ultrastructural observations indicate that the follicular syncytium is synthesizing yolk proteins. Growing follicles incubated in L-[^{3}H]leucine incorporate this amino acid into protein with linear kinetics for 3.5 hr, thus establishing the adequacy of our culture procedure for this time period. Autoradiographic studies show that immediately after a 30-min exposure to L-[^{3}H]leucine, the follicular syncytium is uniformly labeled, whereas the chorion and oocyte are essentially unlabeled. Subsequently, labeled protein accumulates in the apical region of the follicular syncytium, traverses the extracellular space between the follicular syncytium and the oocyte, and, by 3.5 hr, is present in the peripheral yolk bodies of the oocyte. The follicular syncytium thus appears to be the sole heterosynthetic source of yolk protein. Yolk protein does not appear to be taken up into the oocyte by micropinocytosis, as occurs in other systems, but most likely is simply engulfed.

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Physiological Aspects of Oogenesis in Two Species of Sticklebacks, *Gasterosteus aculeatus* and *Apeltes quadracus**

Robin A. Wallace and Kelly Selman†

During May at Woods Hole, female *Gasterosteus aculeatus* periodically produce clutches of 112 ± 19 eggs with an average diameter of 1.31 ± 0.05 mm. Field-collected fish generally have primordial follicles ranging up to 0.56 mm in diameter and a clutch of larger follicles undergoing synchronous growth. The size of oocytes within a growing clutch appears to be random within a population, which indicates that recruitment of clutches is not triggered by a local environmental event. The largest oocytes within the population of primordial follicles have just begun vitellogenesis but are temporarily arrested. All oocytes within follicles larger than 1.1 mm in diameter undergo spontaneous maturation and enlarge to preovulatory size when incubated at 16°C in a simple saline medium. Added deoxycorticosterone can induce similar events in somewhat smaller follicles placed in culture. Thus, *in vivo*, follicles grow from 0.56 to 1.1 mm in diameter by vitellogenesis, and further enlargement is achieved by hydration during steroid-induced maturation. Females carrying follicles in maturational stages also have a new clutch of follicles entering vitellogenic growth from the population of primordial follicles. Injection of human chorionic gonadotropin causes a recruitment of follicles into vitellogenesis regardless of the stage of follicles within the growing clutch. One interpretation of these results is that when vitellogenic follicles reach a diameter of 1.1 mm a surge of

gonadotropin(s) induces the follicle cells to release steroid, which results in oocyte maturation; the same surge also recruits a new clutch of vitellogenic oocytes. Qualitatively similar results were obtained for *Apeltes quadracus*, thus the recruitment phenomenon observed for *G. aculeatus* may be a general feature among sticklebacks.

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Fertilization in the Teleost *Fundulus heteroclitus*

Anna R. Brummett* and James N. Dumont

Eggs of *Fundulus heteroclitus* were fixed at intervals from 1 sec to 15 min following insemination. Chorions were subsequently removed with watchmaker's forceps, and the eggs were further processed for viewing with the scanning and transmission electron microscopes. Sperm penetration, under normal monospermic conditions, was observed and is briefly described.

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Calcium-Induced Dehiscence of Cortical Granules in *Xenopus laevis* Oocytes

T. G. Hollinger,* J. N. Dumont, and R. A. Wallace

Microinjection of 0.1 μg of Ca^{++} into *Xenopus laevis* oocytes induced breakdown of the cortical granules. The cortical granules disappeared in both full grown (Stage VI) and small growing (Stage IV) oocytes. Microinjection of Mg^{++}, K^{+}, or Na^{+} had no effect on cortical granules in either Stage IV or Stage VI oocytes. Small quantities (0.03 μg) of Ca^{++} induced dehiscence of the cortical granules only in proximity to the injection site.

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Oocyte-Follicle Cell Gap Junctions in *Xenopus laevis* and the Effects of Gonadotropin on Their Permeability

Carole L. Browne,* H. Steven Wiley,† and James N. Dumont

Junctions between *Xenopus laevis* oocytes and follicle cells have been identified as gap junctions by the passage of microinjected fluorescent dye from oocytes to follicle cells. The opening and/or assembly of these junctions appears to be regulated by gonadotropins.

*Postdoctoral investigator, subcontract 3322 from the Biology Division of Oak Ridge National Laboratory to the University of Tennessee.

†Predoctoral investigator, Grant 6M-07431-Cell Biology from the National Institute of General Medical Sciences.

Surface Alterations of the Mouse Zona Pellucida and Ovum Following *In Vivo* Fertilization: Correlation with the Cell Cycle

Suzanne Jackowski* and James N. Dumont

The zona pellucida and cell surface of *in vivo* fertilized mouse ova exhibit time-dependent changes which can be detected with the scanning electron microscope. The periods of ovulation, fertilization, and first

cleavage in superovulated C3D2/F_1 hybrids were determined, and times corresponding to G_1, S, G_2, and M were calculated. The zona of a mature unfertilized ovum has a rough texture with deep furrows; at fertilization and thereafter the zona develops a smoother, ropy, and seemingly porous surface. The cell surface of the unfertilized ovum is characterized by uniform microvilli, small blebs, and rounded, moundlike elevations. After fertilization and development to G_1, the ovum loses its blebs but retains the moundlike elevations and microvilli which are now less uniform. As the ovum progresses toward S, it loses the moundlike elevations but retains microvilli in the same density as found in G_1. The ovum in G_2 exhibits smaller but more numerous microvilli which vary considerably in length. Some appear to bifurcate. The fertilized ovum developing through M and G_1 of the two-cell stage exhibits a less dense population of relatively uniform microvilli, periodic blebs and, again, rounded elevations. The data are reminiscent of surface changes associated with the cell cycle in tissue culture cells and indicate a cyclic progression of the *in vivo* fertilized mouse ovum through the first cleavage division to the two-cell stage.

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Toxicity and Teratogenicity of Aromatic Amines to *Xenopus laevis*

James N. Dumont, T. Wayne Schultz,* and Robin D. Jones†

Abstract not available.

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†Undergraduate student, summer 1977, from Douglass College, New Brunswick, New Jersey, grant from the Carnegie Corporation of New York to the University of Tennessee – Oak Ridge Graduate School of Biomedical Sciences.

Toxicity of Selenium to Developing *Xenopus laevis* Embryos

C. L. Browne* and J. N. Dumont

Selenium in the form of sodium selenite is toxic to *Xenopus laevis* embryos and tadpoles continuously exposed to concentrations above 1 ppm. Concentrations of 2 ppm and above result in severe developmental abnormalities and increased mortality. Uptake and loss of radioactive selenium from water are rapid, but depuration is not complete, indicating that some selenium can remain bound by the organism. The facts that selenium is toxic at low levels to *Xenopus* embryos and tadpoles, can cause developmental abnormalities, and accumulates in tissues suggest that increased release of selenium compounds into the environment poses a potential threat to aquatic organisms.

*Postdoctoral investigator, subcontract 3322 from the Biology Division of Oak Ridge National Laboratory to the University of Tennessee.

Uptake, Depuration, and Distribution of Selenium in *Daphnia* and Its Effects on Survival and Ultrastructure

T. Wayne Schultz,* Scott R. Freeman† and James N. Dumont

Selenium is an important essential nutritional trace element which has been shown to provide protection against certain other metal poisoning. However, it is a suspected carcinogen and teratogen. The uptake, depuration, and toxicity of selenium in *Daphnia pulex* have been examined. The LC_{50} at 48 and 96 hr for juvenile animals is 0.6 mg/liter and 0.1 mg/liter, respectively, and for adults it is 1.3 mg/liter and 0.5 mg/liter respectively. Uptake in adult unfed animals is rapid, reaching a maximum at about 12 hr, but

depuration is slow. In fed animals, uptake is slower, reaching a maximum at 96 hr, but initial depuration is followed by a slower prolonged loss. Uptake and depuration rates are related to mode of entry, binding to food and detritus, and metabolism. Localization in cells is primarily in the cytoplasmic compartment although evidence is presented which suggests nucleolar localization. Ultrastructural damage is detected by 16 hr after exposure and is usually confined to the mitochondria. Dense deposits accumulate in the mitochondrial matrices. The nature of these deposits is unknown; they may represent a calcium- or phosphate-selenium complex. With time, the mitochondria degenerate. Nuclear changes, that is, chromatin condensation and vacuolation, are noted. Nerve cells lose their normal complement of neurotubules. It is clear that relatively low concentrations of selenium are toxic to these aquatic organisms and render them incapable of survival in an ecosystem.

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Structure-Toxicity Correlations of Organic Contaminants in Aqueous Coal-Conversion Effluents

T. Wayne Schultz,* Lola M. Kyte, and James N. Dumont

Five series of organic contaminants associated with aqueous coal-conversion effluents were studied to determine the correlation between their known partition coefficients in the octanol-water system and their toxicity to the ciliate *Tetrahymena pyriformis*. In all five series, toxicity increased and solubility decreased with increased alkyl substitution. Compounds containing the equivalent of two or more methyl groups are more toxic than those with one or no alkyl substitutions.

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Reduced Toxicity of an Aqueous Coal-Conversion Effluent Following Waste Disposal Treatment

T. Wayne Schultz,* Stephanie T. Perry,† and James N. Dumont

Abstract not available.

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†Predoctoral investigator, Grant CA 09104 from the National Institutes of Health.

Cytotoxicity of Untreated Coal-Conversion Gasifier Condensate

T. Wayne Schultz,* James N. Dumont, and Lola M. Kyte

The untreated gasifier condensate used in this study is a filtered product water from the Synthane gasification process. To examine possible environmental effects of this product, populations of the ciliate *Tetrahymena pyriformis* were exposed to varying concentrations, and their behavior, respiration, cytology, and growth rates were investigated. Product water concentrations of 1% and less cause little if any behavioral (shape and motility) changes. Concentrations of 2% and greater decrease motility and increase cell lysis. The condensate causes a nonlinear, dose-dependent reduction in oxygen consumption. At concentrations of less than 2%, no alteration in respiration is noted over 300 min. At all concentrations tested, the most striking cytological alterations are in the mitochondrial matrix, which becomes more electron-dense. Cell

membranes are also disrupted, and mucocysts discharge. Population growth is reduced by concentrations of product water as low as 0.2% and is completely inhibited by 1%. The density of test populations plateaus at values inversely related to concentration. Pure phenolic compounds elicit similar responses.

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Cytotoxicity of Untreated Coal Liquefaction Process Water (and a Comparison with Gasification Process Water)

T. Wayne Schultz* and James N. Dumont

The untreated scrubber water from the Char Oil Energy Development coal liquefaction process was examined for pernicious effects on the protozoan *Tetrahymena pyriformis*. Cells were exposed to varying concentrations of process water; their behavior, oxygen uptake, and population growth were monitored. Reduction in cell number and motility are directly related to both concentration and length of exposure with concentrations up to 5% and exposures up to 24 hr. The product water causes a nonlinear, dose-dependent reduction in oxygen uptake over a 2 to 5% concentration range. Population growth is reduced by concentrations as low as 0.2% process water. Both the growth rate and the final density of the test populations are inversely related to concentration over the range of 0.2 to 1%. Comparisons with the coal gasification product water are discussed.

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Cytotoxicity of Synthetic Fuel Products on *Tetrahymena pyriformis*. III. Shale Oil Retort Water

T. Wayne Schultz,* James N. Dumont, and Lola M. Kyte

Shale oil retort water was obtained by centrifuging an oil/water emulsion produced by oil shale retorting. The ciliated protozoa *Tetrahymena pyriformis* strain GL-C, syngen 1 was exposed to varying concentrations of shale oil retort water, and its behavior, cytology, respiration, and growth rates were investigated. Concentrations of 2, 1, and 0.5% retort water cause an initial increase in motility, while with longer exposure motility decreases. Toxicant concentrations of 3, 4, and 5% all cause a decrease in motility. Cell lysis is directly related to concentration; after 24 hr population densities are 0, 10, and 25% of controls for 2, 1, and 0.5% product water respectively. Oxygen consumption parallels the motility pattern in that it increases initially at lower concentrations but begins to decrease with extended lengths of exposure. At higher concentrations oxygen uptake falls off rapidly. The most striking cytological alteration of cells exposed to the toxicant occurs in the membranes, although alterations of the mucocysts and glycogen content are also observed. No mitochondrial changes are observed. Population growth is affected at much lower concentrations than the other test parameters. The growth of test populations plateaus at values inversely related to concentration, with concentrations less than 0.4% having no effect on the rate of growth.

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MOLECULAR AND CELLULAR SCIENCES SECTION

Although our research interests are varied, we share a common bond in our conviction that a more comprehensive understanding of fundamental life processes is required to solve complex biological problems associated with human exposure to pollutants (chemicals and radiation) that can result from the production of energy. These pollutants are potentially acutely toxic, carcinogenic, mutagenic, or teratogenic. Since the clinical manifestations of the latter three effects have as their origin alteration of the genetic material, much of our work centers around the chemical and physical characterization of DNA and chromatin, mechanisms of replication and transcription, and elucidation of repair processes that reverse potentially deleterious modifications of DNA. We are also active in such diverse areas as molecular genetics of tRNA function, processing of RNA in normal and cancerous cells, radiation biology, enzymic mechanisms, membrane function and turnover, developmental biology, and cryobiology.

We stress that studies within our Section be coordinated with the goals of the entire Division. Many of our research endeavors are interwoven with those of the other two Sections in the form of collaborative investigations.

Proteolytic Inactivation and Coordinate Protection of the *arom* Enzyme Conjugate of *Neurospora**

A. Vitto† and F. H. Gaertner

The *arom* enzyme system of *Neurospora crassa* is a pentafunctional enzyme conjugate which catalyzes five steps in the synthesis of the aromatic amino acids. The occurrence of five enzymes on a single polypeptide chain has provided us with a unique opportunity to examine the response of a multidomained protein structure to limited proteolytic attack. We have found that the first substrate, 3-deoxy-D-arabinoheptulosonate 7-phosphate (DAHP), coordinately protects all five activities from inactivation by three different protease preparations. The order of enzyme inactivation remains the same regardless of the protease used, and the two most sensitive activities are both regulatory enzymes. Hence the concerted actions of coordinate protection and selective enzyme inactivation may provide a mechanism for regulating the *arom* pathway. We have also identified two components of "first substrate protection." Specifically, DAHP protects from proteolysis per se and also maintains native structure once proteolysis has taken place. The rather extensive proteolysis that takes place prior to loss of native structure and the dual component protection by the first substrate suggest a process of structural change during which the conjugate protein "ages." This protein senescence and the presence of first substrate may be critical to the viability of the *arom* pathway, especially during later stages of growth.

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Proteolytic Inactivation of a Pentafunctional Enzyme Conjugate: Coordinate Protection by the First Substrate*

A. Vitto† and F. H. Gaertner

The *arom* pentafunctional enzyme conjugate of *Neurospora crassa* was exposed to trypsin, chymotrypsin, or a protease preparation from *Neurospora* in the presence and absence of the first substrate, 3-deoxy-D-arabinoheptulosonate 7-phosphate. It was found that the first substrate coordinately protects all five activities from proteolytic inactivation, which indicates a conformational change induced by this compound. In addition, the data presented are consistent with the "domain" theory of conjugate structure. It is also argued that coordinate protection may be of physiological significance.

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The *arom* Enzyme Conjugate of *Neurospora:* Multiple Proteases and Subunit Artifacts*

F. H. Gaertner, A. Vitto, D. Allison, K. W. Cole, and G. E. Spady

The *arom* enzyme conjugate of *Neurospora crassa* is a multifunctional protein consisting of five enzymes that catalyze a sequence of reactions leading to the biosynthesis of the aromatic amino acids. Previously, it was believed that this multienzyme system consisted of a set of physically associated but noncovalently linked enzymes. We now have good evidence that all five activities reside on a single polypeptide chain, and electron micrographs confirm that the enzyme conjugate is a dimer of this multifunctional polypeptide. The enzyme conjugate is very susceptible to proteolytic attack. However, several discrete proteolytic clips can occur before the system loses either its overall native structure or any one of its five activities. The uncontrolled action of a multiplicity of resident proteases in *N. crassa* during the extraction and purification of the conjugate led us to the false conclusion that this enzyme system consisted of noncovalently linked enzymes. Which of the many proteases that appear to be present in *N. crassa* are primarily involved in forming the artifactual subunits remains to be determined.

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Catalytic and Structural Properties of the Pentafunctional *arom* Enzyme Conjugate*

F. H. Gaertner

The *arom* enzyme conjugate exhibits several unique catalytic and structural features which may provide the system with various physiological advantages. The presence of all five activities on a single polypeptide chain may be important in both the coordinate activation and coordinate protection of the *arom* conjugate. The physical association of the enzymes may (*a*) enable the system to respond rapidly with little or no transient to changes in metabolic flux, (*b*) provide an inherent increase in the catalytic capacity of the individual enzymes, and (*c*) allow for the channeling of intermediate substrates, thereby sequestering them from a competing catabolic pathway.

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Unique Catalytic Properties of Enzyme Clusters

F. H. Gaertner

Physically associated multienzyme systems (enzyme clusters) have the potential of expressing unique catalytic properties in contrast to their unassociated counterparts. Evidence is accumulating which suggests that many of the enzymes in cells are organized in some manner and that at least some of the conceived unique catalytic properties are expressed.

Artifactual Subunit Structure in the *arom* Enzyme Conjugate: Evidence for a Multiplicity of Proteases in *Neurospora crassa**

G. E. Spady† and F. H. Gaertner

Proteolytic contaminants cause an artifactual subunit structure in the *arom* enzyme conjugate in *Neurospora crassa*. In an attempt to resolve this problem and to find conditions favorable for protease-free isolation of the *arom* conjugate, we examined the endogenous proteases of early logarithmic and stationary growth phases of *N. crassa*. Each growth stage was fractionated three times with ammonium sulfate and then separated by ion exchange chromatography. Each chromatographic fraction was assayed at acidic, neutral, and alkaline pH with Azocoll, a highly sensitive, general substrate. In addition, the chromatographic fractions were assayed in the presence of several natural inhibitors or commercial inhibitors to aid in defining the many proteolytic activities expressed in the presence of Azocoll. Much of this activity was cryptic and was assayable only after preparations had been stored. We conclude that there are many more intracellular proteases in *N. crassa* than the five thus far reported. In addition, we suggest that other organisms also may have a similarly large number of unrecognized proteases and that these enzymes may be involved in a heretofore unrecognized complexity in the regulation of development and enzyme function.

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Kynureninase-type Enzymes from Two Strains of *Xanthomonas pruni**

A. S. Shetty† and F. H. Gaertner

Xanthomonas pruni is the only bacterium known to have the capacity to synthesize nicotinamide adenine dinucleotide (NAD) from L-tryptophan. This organism was shown to carry a single kynureninase-type activity, which is inducible by L-tryptophan and has a K_m for L-kynurenine (16–27 μm) approximately 0.1 the K_m for L-3-hydroxykynurenine. Hence on a purely biochemical basis, this enzyme can be properly characterized as a kynureninase rather than a hydroxykynureninase. However, since this enzyme appears to be the only kynureninase-type activity in *X. pruni*, we believe that it is probably a physiologically bifunctional enzyme involved in two pathways: one catalyzing the degradation of L-tryptophan to anthranilate, the other catalyzing the biosynthesis of NAD from L-3-hydroxykynurenine. These results suggest the possibility that *X. pruni* may represent an extant example of one of the major transitions in the evolution of the aerobic L-tryptophan to NAD pathway.

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A Cryobiological Method for the Enrichment of Fungal Mutants

James L. Leef* and Frank H. Gaertner

Auxotrophs of *Neurospora crassa* can be isolated from a mixture of wild-type and mutant conidia by using the differential killing effects of freezing. The technique involves incubating conidia under conditions in which only wild-type organisms will germinate and then subjecting the conidial mixture to freezing and thawing at cooling and warming rates known to be lethal to germinated conidia. This single-step freeze-thaw procedure provides ~1000-fold enrichment of auxotrophs. When the spores are frozen slowly, killing of germinated conidia is accomplished primarily by the effects of dehydration; if they are frozen rapidly, killing is caused by the formation of intracellular ice. It is also possible to obtain enrichment of mutants by dehydration without freezing.

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Sequences of Two Active-Site Peptides from Spinach Ribulosebisphosphate Carboxylase/Oxygenase

Claude D. Stringer and Fred C. Hartman

Two tryptic peptides from spinach ribulosebisphosphate carboxylase/oxygenase that contain the essential lysyl residues derivatized by the affinity label 3-bromo-1,4-dihydroxy-2-butanone 1,4-bisphosphate were subjected to sequence anlyses. The sequences of these peptides are -Tyr-Gly-Arg-Pro-Leu-Leu-Gly-Cys-Thr-Ile-Lys-Pro-Lys- and -Leu-Ser-Gly-Gly-Asp-His-Ile-His-Ser-Gly-Thr-Val-Val-Gly-Lys-Leu-Glu-Gly-Glu-Arg- respectively. The reagent moiety is covalently attached to the internal lysyl residue in each peptide.

Identification of Essential Lysyl and Cysteinyl Residues in Spinach Ribulosebisphosphate Carboxylase/Oxygenase Modified by the Affinity Label *N*-Bromoacetylethanolamine Phosphate

John V. Schloss,* Claude D. Stringer, and Fred C. Hartman

We reported earlier that *N*-bromoacetylethanolamine phosphate is an affinity label for spinach ribulosebisphosphate carboxylase/oxygenase. We now show inactivation to be correlated directly with the alkylation either of a single lysyl residue (in the presence of Mg^{2+}) or of two different cysteinyl residues (in the absence of Mg^{2+}), consistent with the likelihood that these residues are located in the active-site region. This proposition is further supported by the demonstration that the residues are protected from alkylation by substrate, a competitive inhibitor, or the transition state analog 2-carboxyribitol bisphosphate. Tryptic peptides that contain the modified residues have been isolated and sequenced. One of the two cysteinyl residues subject to alkylation is only three residues distant in sequence from the lysyl residue modified by bromoacetylethanolamine phosphate. This lysyl residue is identical to one of the two lysyl residues alkylated by the previously described affinity label, 3-bromo-1, 4-dihydroxy-2-butanone 1,4-bisphosphate.

*Predoctoral investigator, Grant GM 1974 from the National Institute of General Medical Sciences.

Inactivation of Ribulosebisphosphate Carboxylase by Modification of Arginyl Residues with Phenylglyoxal

John V. Schloss,* I. Lucile Norton, Claude D. Stringer, and Fred C. Hartman

Phenylglyoxal rapidly and completely inactivates spinach and *Rhodospirillum rubrum* ribulosebisphosphate carboxylases. Inactivation exhibits pseudo-first-order kinetics and a reaction order of approximately one for both enzymes, suggesting that modification of a single residue per protomeric unit suffices for inactivation. Loss of enzymic activity is directly proportional to incorporation of [^{14}C]phenylglyoxal until only 30% of the initial activity remains.

For both enzymes, extrapolation of incorporation to 100% inactivation yields 4–5 mol of [^{14}C]phenylglyoxal per mole of protomer. Amino acid analyses confirm the expected 2:1 stoichiometry between phenylglyoxal incorporation and arginyl modification and suggest that other kinds of amino acid residues are not modified. (Thus, inactivation correlates with modification of 2 to 3 arginyl residues per protomer.) The substrate ribulosebisphosphate and some competitive inhibitors reduce the rates of inactivation of the carboxylases and prevent modification of about 0.5 to 1.0 arginyl residue per protomer. Inactivation is therefore a consequence of modification of a small number of residues out of the 35 and 29 total arginyl residues in spinach and *R. rubrum* carboxylases respectively.

*Predoctoral investigator, Grant GM 1974 from the National Institute of General Medical Sciences.

Isolation, Characterization, and Crystallization of Ribulosebisphosphate Carboxylase from Autotrophically Grown *Rhodospirillum rubrum*

John V. Schloss,* E. F. Phares, Mary V. Long, I. Lucile Norton, Claude D. Stringer, and Fred C. Hartman

Serial culture of *Rhodospirillum rubrum* with 2% CO_2 in H_2 as the exclusive carbon source resulted in a rather large fraction of the soluble protein (>40%) being comprised of ribulosebisphosphate carboxylase (about sixfold higher than the highest value previously reported). Isolation of the enzyme from these cells revealed that it has similar physical and kinetic properties to those previously described for the enzyme derived from cells grown on butyrate. Notably, the small subunit (which is a constituent of the carboxylase from eukaryotes and most prokaryotes) was absent in the enzyme from autotrophically grown *R. rubrum*. Edman degradation of the purified enzyme revealed that the NH_2-terminus is free (in contrast to the catalytic subunit of the carboxylase from eukaryotes) and that the NH_2-terminal sequence is Met-Asp-Gln-Ser-Ser-Arg-Tyr-Val-Asn-Leu-Ala-Leu-Lys-Glu-Glu-Asp-Leu-Ile-Ala-Gly-Gly-Glx-His-Val-Leu-. Crystals of the enzyme were readily obtained by dialysis against distilled water. The availability of large quantities of this enzyme provides an impetus for sequence and X-ray crystallographic studies.

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Synthesis and Characterization of *cis*- and *trans*-2,3-Epoxybutane-1,4-Diol 1,4-Bisphosphate, Potential Affinity Labels for Enzymes that Bind Sugar Bisphosphates

John V. Schloss* and Fred C. Hartman

cis- and *trans*-2,3-Epoxybutane-1,4-diol 1,4-bisphosphate, which can be considered reactive analogs of several sugar bisphosphates, have been synthesized in a continuing effort to develop new and diverse affinity labeling reagents for enzymes that bind phosphorylated substrates. *cis*-2,3-Epoxybutane-1,4-diol was obtained by epoxidation of commercially available *cis*-2-butene-1,4-diol with *m*-chloroperbenzoic acid; the trans epoxide was obtained by reduction of 2-butyne-1,4-diol with $LiAlH_4$ followed by epoxidation with *m*-chloroperbenzoic acid. The diols were phosphorylated with diphenyl chlorophosphate, and the phenyl blocking groups were then removed by platinum-catalyzed hydrogenation. By the criterion of their reaction with the sulfhydryl group of glutathione, the phosphorylated epoxides are 6000 times less electrophilic than the previously described and structurally similar reagent 3-bromo-1,4-dihydroxy-2-butanone 1,4-bisphosphate.

*Predoctoral investigator, Grant GM 1974 from the National Institute of General Medical Sciences.

Attempts to Apply Affinity Labeling Techniques to Ribulosebisphosphate Carboxylase/Oxygenase

Fred C. Hartman, I. Lucile Norton, Claude D. Stringer, and John V. Schloss*

Abstract not available.

*Predoctoral investigator, Grant GM 1974 from the National Institute of General Medical Sciences.

Reactive Phosphate Esters as Affinity Labels for Enzymes of Carbohydrate Metabolism

Fred C. Hartman, I. Lucile Norton, Claude D. Stringer, and John V. Schloss

Abstract not available.

Effect of $Mg \cdot CTP^{2-}$ and $Mg \cdot GTP^{2-}$ on the 3′→5′ Exonuclease Activity of T5-Induced DNA Polymerase

Shishir K. Das* and Robert K. Fujimura

$Mg \cdot CTP^{2-}$ and $Mg \cdot GTP^{2-}$ inhibit the 3′→5′ exonuclease action by T5-induced DNA polymerase on natural DNA. $Mg \cdot ATP^{2-}$ and $Mg \cdot UTP^{2-}$ do not produce any effect when similar concentrations are used. Neither $Mg \cdot CTP^{2-}$ nor $Mg \cdot GTP^{2-}$ is utilized as substrate. The inhibition produced by these nucleotides seems to be template-specific and noncompetitive with respect to the DNA substrate.

*Research assistantship from the University of Tennessee.

Mechanism of T5-Induced DNA Polymerase: Processiveness and Proofreading Function of the 3′→5′ Exonuclease Activity*

Shishir K. Das†

T5-induced DNA polymerase has an associated 3′→5′ exonuclease activity. Both single-strand and duplex DNA are hydrolyzed by this enzyme in a processive manner. This is indicated by the results of polymer-challenge experiments and experiments utilizing product analysis techniques. Due to the processive mode of hydrolysis, the kinetics of label release from the 3′-terminally labeled oligonucleotide substrates, annealed to complementary homopolymers, are nonlinear. In the case of both single-strand and duplex DNA substrates, hydrolysis seems to continue at best, up to the point where the enzyme is five or six nucleotides away from the 5′-end.

The frequency of misincorporation as measured *in vitro* with synthetic substrates is ~1/10,000. In the presence of Mn^{2+} this value is increased by a factor of 4. The enzyme does carry out mismatch repair, as evidenced by experiments with primer molecules carrying improper base residues at the 3′-OH terminus. Control experiments with complementary base residues at the 3′-end indicate that extensive removal of terminal residue takes place in the presence of dNTPs only when such residues are "improper" in the Watson-Crick sense.

*Third in a series on mechanism of T5-induced DNA polymerase. Papers I and II may be found in *J. Biol. Chem.* **252**, 8700–8712 (1977).

†Research assistantship from the University of Tennessee.

Processiveness of DNA Polymerases: A Comparative Study Using a Simple Procedure

Shishir K. Das* and Robert K. Fujimura

In this communication we describe a simple procedure for analyzing the processiveness of DNA polymerases in general. By choosing conditions for which the number of incorporations per primer is <1, we have reduced the probability of a primer molecule being utilized by the enzyme more than once. The primer-template used was poly$(dA)_{300}$:oligo$(dT)_{10}$, and the product was isolated by oligo(dT)-cellulose chromatography. The number of dTMP residues added per association was determined from the [^{3}H]dThd + [3′-^{3}H]dTMP/[^{3}H]dThd ratio of the product after its digestion by micrococcal nuclease and spleen phosphodiesterase.

Using this procedure, we have found that *Escherichia coli* DNA polymerase I, T4 DNA polymerase, and calf thymus α- and β-DNA polymerase are "quasi-processive." Most of these enzymes add on the average ~10–15 nucleotides before dissociating from the template. T5 DNA polymerase, on the other hand, is processive; that is, it continues to replicate a given template until it is very close to the 5′-end of the template. With "nicked-DNA-like" poly(dA):oligo(dT), the processiveness of *E. coli* DNA polymerase I is increased 2- to 2.5-fold. The significance of this increase in determining the "patch size" during DNA repair is discussed.

*Research assistantship from the University of Tennessee.

Origin and Direction of Synthesis of Replicative Form DNA of Coliphage M13

Cathrine E. Snyder and Sankar Mitra

We studied the replication of M13 replicative-form (RF) DNA in the absence of single-strand DNA synthesis by examining the amount of pulse-labeled M13 am5 DNA in the different regions of the molecule by restriction fragment analysis. The technique is based on preferential pulse-labeling of the termini of nascent molecules. The gradients we observed using a 60-sec, or shorter, pulse at 25° were distinctly "v"-shaped. It is possible to interpret our data in two different ways: We can assume that the gradients are composites of two gradients of replication, proceeding unidirectionally but with opposite handedness. On the other hand, an alternative interpretation of a "v"-shaped gradient is to assume that it is the result of bidirectional replication similar to that in SV40. According to this model, we found that RF → RF replication has the same terminus as unidirectional parental RF and single-strand DNA synthesis, but the origin is at a site 180° from the terminus on the circular molecule. An attractive feature of the bidirectional mechanism involving Θ structures is the prediction of several facets of M13 RF DNA replication that are in agreement with experimental observations.

A Mechanism for the Organotropism of Nitrosamines in Rats*

Cathrine E. Snyder

Lipophilic cyclic nitrosamines are excreted by the lung of rats given small oral doses. These data and other data from earlier testing of metabolism and carcinogenicity lead to the conclusion that nitrosamines are carcinogenic to organs related to the respiratory system because of their differential accumulation in the target organ following incomplete detoxification by the liver. The bladder may be a target for nitrosamine carcinogenicity after accumulation of intermediate hydrophilic metabolic products, and the liver is a target when it detoxifies the carcinogenic compound completely. The toxicity and carcinogenicity of nitrosamines are directly related, and the indications are that both may be the result of oxidative metabolism.

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Comparison of Specific Binding Sites for *Escherichia coli* RNA Polymerase with Naturally Occurring Hairpin Regions in Single-Strand DNA of Coliphage M13

Salil K. Niyogi and Sankar Mitra

Escherichia coli RNA polymerase binds specifically to the single-strand circular DNA of coliphage M13 in the presence of a saturating concentration of the bacterial DNA binding protein, presumably as an essential step in the synthesis of the RNA primer required for synthesizing the complementary DNA strand in parental replicative-form DNA. The RNA polymerase-protected DNA regions were isolated after extensive digestion with pancreatic DNase, endonuclease Sl of *Aspergillus oryzae*, and exonuclease I of *E. coli.* The physicochemical properties of the RNA polymerase-protected segments (called PI and PII) were compared with those of the naturally occurring hairpin regions (termed core I and core II) in M13 DNA. PI and PII were approximately 80 and 45 nucleotides long, whereas core I and core II were 60 and 44 nucleotides long, respectively, under denaturing conditions. Furthermore, even though all the DNA fragments had (G+C)-contents higher than that of the intact M13 DNA, PI and PII maintained a predominantly single-strand character, unlike core I and core II, as judged by their susceptibility to single-strand-specific nucleases. However, core I and core II, although generally nondenaturable, did not show a perfect double-strand character, as judged by the lack of complete base complementarity, elution from hydroxyapatite, and susceptibility to exonuclease I upon pretreatment with Sl endonuclease followed by heat denaturation. Although all of the fragments could be located predominantly, but not exclusively, in a specific region (endo R•*Hpa*II-F fragment) in the M13 DNA, these results suggest that RNA polymerase does not bind directly to the hairpin regions in M13 DNA, although there could be an overlap between the latter and the RNA polymerase-bound regions.

Isolation and Characterization of Naturally Occurring Hairpin Structures in Single-Strand DNA of Coliphage M13

Salil K. Niyogi and Sankar Mitra

With precise conditions of digestion with single-strand-specific nucleases, namely, endonuclease Sl of *Aspergillus oryzae* and exonuclease I of *Escherichia coli*, nuclease-resistant DNA cores can be obtained reproducibly from single-strand M13 DNA. The DNA cores are composed almost exclusively of two sizes (60 and 44 nucleotides long). These have high (G+C)-contents relative to that of intact M13 DNA and arise from restricted regions of the M13 genome. The resistance of these fragments to single-strand-specific nucleases and their nondenaturability strongly suggest the presence of double-strand segments in these core pieces. That the core pieces are only partially double strand is shown by their lack of complete base complementarity and their pattern of elution from hydroxyapatite.

Interspersion of Highly Repetitive DNA with Single Copy DNA in the Genome of the Red Crab *Geryon quinquedens*

Nelwyn T. Christie* and Dorothy M. Skinner

Kinetic analysis of the reassociation of 420-nucleotide-long (NT) fragments has shown that essentially all of the repetitive sequences of the DNA of the red crab *Geryon quinquedens* are highly repetitive. There are indetectable amounts of low and intermediate repetitive DNAs. Though atypical of eukaryotes, this pattern has also been observed in all other brachyurans (true crabs) studied. The major repetitive component accounts for 29% of the genome and is subdivided into short runs of 300 NT and longer runs of greater than 1200 NT. A minor component with an average sequence length of 400 NT accounts for about 4% of the genome. Both components are present at frequencies commonly observed for satellite DNAs: 8.3×10^4 and 4×10^6 copies respectively. Unique among eukaryotes, the organization of the genome includes single-copy DNA contiguous to short runs (~300 NT) of both repetitive components. Subsets of the repetitive DNA have been isolated either by restriction endonuclease digestion or by centrifugation in Ag^+ or Hg^{2+}/Cs_2SO_4 density gradients.

*Predoctoral investigator, Grant GM 1974 from the National Institute of General Medical Sciences.

Changes in Ecdysteroids During Embryogenesis of the Blue Crab *Callinectes sapidus* Rathburn

John F. McCarthy* and Dorothy M. Skinner

Total ecdysteroid titers [estimated by radioimmunoassay (RIA)] in eggs of the blue crab increased from ~6 ng/g in the immature egg to a maximum of ~500 ng/g in maturing embryos; titers dropped to ~300 ng/g in prehatch embryos. High-pressure reverse phase chromatography of the egg extracts resolved five RIA-active components. α-Ecdysone and the polar conjugate of β-ecdysone were present in low quantities. The concentration of β-ecdysone increased during embryogenesis to a maximum of ~160 ng/g in maturing eggs and decreased only slightly in prehatch embryos. Two unidentified components were also detected, and the changes in their concentrations were estimated. One, an apolar component (peak III), accounted for as much as 63% of the total RIA activity as the eggs matured. The estimated concentration of this component increased from 120 ng/g in early embryos to 770 ng/g in maturing eggs, then decreased by 50% in prehatch embryos. The level of the more polar component (peak II) increased from 7.5 to 75 ng/g as the embryos developed.

The increase in the concentration of ecdysteroids during embryogenesis indicates that crab embryos have the capacity to synthesize ecdysteroids and suggests that these hormones may have a physiological role in the embryonic development of crustaceans.

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Metabolism of α-Ecdysone in Intermolt Land Crabs, *Gecarcinus lateralis*

John F. McCarthy* and Dorothy M. Skinner

The metabolic fate of α-ecdysone was examined in intermolt crabs to elucidate the biochemical pathways as well as the dynamics of ecdysteroid metabolism. At 1–24 hr after injection of α-[^{3}H]ecdysone, serum was sampled and epidermis, midgut gland, hindgut, and carcass were dissected; ecdysone metabolites were extracted from each tissue and separated by reverse phase chromatography. Ecdysone metabolism proceeded through (*a*) sequential hydroxylation at C-20 and C-26 and (*b*) formation of glycosidic conjugates of the free ecdysteroids.

The conversion of α- to β-ecdysone was very rapid; within 1–4 hr over 50% of the radioactivity was present as β-ecdysone. Further hydroxylation of β-ecdysone to 20,26-dihydroxyecdysone was less rapid but continued over the experimental period. The three ecdysteroids were conjugated, probably as β-glycosides. These conjugates comprised 20% of the total radioactivity in the crab, over half of which was concentrated in the midgut gland and hindgut. Free and conjugated ecdysteroids as well as an extremely polar material, which was resistant to enzymatic hydrolysis, were excreted in both the urine and feces.

The rate of elimination of both total radioactivity and β-ecdysone was fairly rapid but varied among the different tissues. The rate of elimination from the epidermis was very rapid ($k \sim 0.08\ hr^{-1}$). Loss from the carcass and from the whole crab was slower ($k \sim 0.02\ hr^{-1}$), perhaps due to sequestration of some of the metabolites in tissues which are inactive in the metabolism of ecdysteroids.

*Postdoctoral investigator, Institutional National Research Service Award 5 T32 AG00028 from the National Institutes of Health.

An Exoribonuclease from *Saccharomyces cerevisiae:* Effect of Modifications of 5′-End Groups on the Hydrolysis of Substrates to 5′-Mononucleotides

Audrey Stevens

Using polyriboadenylic acid [poly(A)] as a substrate, an exoribonuclease has been purified from the high-salt wash of ribosomes of *Saccharomyces cerevisiae*. The product of the reaction of the exoribonuclease is adenosine 5′-monophosphate (AMP). Hydrolysis of $[^3H](pA)_3[^{14}C](pA)_n$ shows that both labels are released at the same rate, suggesting that the enzyme acts in a processive manner. Removal of the terminal phosphate of poly(A) with alkaline phosphatase reduces the rate of hydrolysis by 80%. Treatment of the terminally dephosphorylated poly(A) with polynucleotide kinase restores the activity. Two 5′-capped mRNAs have been tested, and they are hydrolyzed slowly, if at all, by the enzyme. In contrast, phage T4 mRNA, ribosomal RNA, and encephalomyocarditis viral RNA are hydrolyzed at greater than 50% of the rate of poly(A).

On the Question of Compartmentalization of the Nucleotide Pool

Joseph X. Khym, M. Helen Jones, William H. Lee, James D. Regan, and Elliot Volkin

Plagemann observed that a fourfold expansion of the pyrimidine ribonucleotide pool of Novikoff rat hepatoma cells by high concentrations of exogenous uridine does not reduce the subsequent rate or extent of [^{3}H]uridine incorporation into RNA, compared to control, unexpanded cells. On the basis of these and related data, he concludes that the pool is compartmentalized into virtually independent cytoplasmic and nuclear pools and that only the small nuclear pool serves as the functional one for RNA (except mitochondrial) synthesis. This idea has received support from some investigators using different approaches; however, others interpret their observations to mean that RNA is synthesized from the total cellular ribonucleotide pool.

We have extended a modified version of the Plagemann experiments to three other well-established cell lines, to fibroblast cultures from three different sources, and to the Novikoff and HeLa lines. Of these, six different cell cultures demonstrated a subsequent reduced capacity to incorporate [^{3}H]uridine proportional to the extent of the prior expansion of their pyrimidine pools, suggesting a single functional pool. In contrast, Novikoff and to a lesser extent HeLa exhibited labeling characteristics as if they contained a functional pool separate from the total pool. In our hands Novikoff and HeLa, however, seem to have a greater facility than the other cell types for reducing their expanded pools when placed back in low concentrations of uridine, possibly complicating interpretation of this kind of experiment. On the other hand, the subsequent labeling of the uridine phosphate (mostly UTP) pool in the other three cell lines was not reduced to the level expected on the basis of the extent of prior UTP expansion, which is indicative of some degree of compartmentalization in these cells as well. From these data we propose, as a unified interpretation for all cells in culture, that there is compartmentalization of nuclear and cytoplasmic ribonucleotide pools in all cells, but in most kinds of cells there is a rapid equilibrium between these pools. In the Novikoff hepatoma the pools equilibrate slowly; in HeLa they equilibrate at an intermediate rate.

In addition, we observed that although label seldom decreases in total RNA after a chase in excess uridine, analysis of the radioactivity in the RNA nucleotides reveals that this is caused to a large extent by the increase in RNA-CMP as a result of the uridine to cytidine time-dependent metabolic conversion. Substantial RNA turnover in all cell types can always be demonstrated by the loss in RNA-UMP radioactivity.

M^7 Guanosine in Transfer RNA of *Escherichia coli*

Lee Shugart and Barbara Chastain

The nucleoside content of six different individual tRNAs was reinvestigated. Particular emphasis was placed on obtaining an accurate, quantitative estimation of the amount of the minor nucleoside M^7 guanosine in

each tRNA. The observation that this nucleoside occurs at less than one full residue in $tRNA^{Phe}_{E.\ coli}$ and $tRNA^{Arg}_{E.\ coli}$ is explained by a possible base-pairing at positions 44 and 49 in these tRNAs, which produces a conformational structure in the extra arm region that is unsuitable for methylation.

Kinetic Studies of *Escherichia coli* Transfer RNA (Uracil-5-)-Methyltransferase

Lee Shugart

The kinetic mechanism of a semipurified tRNA (uracil-5-)methyltransferase (EC 2.1.1.35) preparation obtained from *Escherichia coli* has been studied at pH 9.0 in the presence and absence of products. The initial velocity and product inhibition patterns remain consistent with a random order of addition of adenosylmethione and transfer RNA to separate and independent binding sites on the enzyme. Values have been determined for the Michaelis and product inhibitor constants.

Regulation of Active Amino Acid Transport by Growth-Related Changes in Membrane Potential in a Human Fibroblast*

Mitchel L. Villereal† and John S. Cook

Indirect measurements of membrane potential in human diploid fibroblast cells indicate that there is a growth-related difference in membrane potential between subconfluent, growing cells (–49 mV) and serum-deprived, quiescent cells (–22 mV). The elevated membrane potential in growing cells appears to be solely responsible for the twofold higher α-aminoisobutyric acid accumulating capacity that we observed previously in growing cells, as compared to quiescent cells. When the membrane potentials of quiescent and growing cells are set equal, using valinomycin and varying $[K^+]_o$, α-aminoisobutyric acid accumulation is dependent on the magnitude of the potential but is independent of the growth state of the cell. Initial α-aminoisobutyric acid influx measurements in growing and quiescent cells show that when cells become quiescent the K_m increases and the V_{max} of the transport system decreases. For growing cells, K_m = 1.2 ± 0.09 m*M* and V_{max} = 15.6 ± 0.9 μmol/g protein/min. For quiescent cells, K_m = 2.1 ± 0.18 m*M* and V_{max} = 8.5 ± 0.8 μmol/g protein/min. Serum stimulation of quiescent cells for 1 hr results in a decrease in K_m (to 1.2 ± 0.13 m*M*) and no change in V_{max} (7.8 ± 0.8 μmol/g protein/min). Hyperpolarization of quiescent cells by valinomycin also induces a decrease in K_m (to 1.2 ± 0.13 m*M*) without a change in V_{max}. This suggests that the immediate response of initial α-aminoisobutyric acid transport to serum stimulation is mediated by a membrane hyperpolarization. These data indicate that both the accumulation of α-aminoisobutyric acid in human diploid fibroblast cells and the K_m for its transport are sensitive to experimentally induced changes in membrane potential and, furthermore, that the observed growth-related difference in membrane potential between growing and quiescent cells can account for their growth-associated differences in α-aminoisobutyric acid accumulation and K_m.

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†Postdoctoral investigator, Carcinogenesis Training Grant CA 05296 from the National Cancer Institute.

Specific Binding of Phloretin and Cytochalasin B in Relation to Inhibition of Hexose Transport in a Human Fibroblast*

Donald W. Salter† and John S. Cook

The binding to cells and the inhibition of hexose transport have been compared for cytochalasin B and phloretin in a strain of diploid human fibroblasts designated HSWP. [^{3}H]Cytochalasin B binding to whole cells is noncompetitive with either D- or L-glucose. A Scatchard plot can be fit to the data assuming two classes of binding sites. In double-label experiments where [^{14}C]deoxyglucose uptake is assessed simultaneously with binding, the apparent K_i for cytochalasin B inhibition of hexose transport (0.1–0.6 μ*M*)

corresponds to the K_d for its high-affinity binding. The number of high-affinity binding sites, 1.1×10^6 cell^{-1}, is taken as an upper limit for the number of hexose transporters. Phloretin binds in a pH-dependent manner, which suggests that only the protonated (uncharged) form is binding to the cells; it also binds in a time-dependent manner, suggesting that this uncharged form penetrates the cells. Phloretin also shows two-component binding with the K_d (10–15 μM) of the higher affinity site corresponding to the apparent K_i for inhibition of hexose transport. There are 3×10^9 to 5×10^9 high-affinity sites per cell. Phloretin binding to these sites is competitively displaced by diethylstibestrol and quercetin at concentrations corresponding to the apparent K_is for hexose-uptake inhibition by these compounds. Phloridzin also displaces phloretin but only when the former is at concentrations tenfold greater than required for inhibition of hexose uptake; the two effects of phloridzin may be independent. Phloretin binding is not competitively displaced by cytochalasin B, or vice versa. We conclude that phloretin acts not by interacting directly with the transporter, but by intercalating in and perturbing the bilayer in which the transporters are anchored and must function.

*Research sponsored jointly by the National Cancer Institute under Interagency Agreement 40-5-63 and the Division of Biomedical and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with Union Carbide Corporation.

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Characterization of HeLa 5′-Nucleotidase: A Stable Plasma Membrane Marker

Emily T. Brake, Peter C. Will,* and John S. Cook

5′-Nucleotidase, assayed as 5′-AMPase, has been extensively characterized and established as a stable, quantitative plasma membrane marker in HeLa S3 cells. The membrane 5′-AMPase has a K_m of 7.0 μM. Relative affinities of the other 5′-mononucleotides for the enzyme are 5′-GMP > 5′-TMP > 5′-UMP > 5′-CMP. There are activity optima at pH 7 and 10; the latter is Mg^{2+} dependent. The membrane preparations have a small amount of acid phosphatase activity that is distinct from 5′-AMPase activity but no alkaline phosphatase. AOPCP, ADP, and ATP are strongly inhibitory. Mg^{2+}, Ca^{2+}, or Co^{2+} additions do not affect the pH 7.0 activity; Mn^{2+} activates slightly, while Zn^{2+}, Cu^{2+}, and Ni^{2+} are inhibitory. EDTA slowly inactivates, but removal of the EDTA without the addition of divalent cations restores activity. The inactivation is also substantially reversed by Co^{2+} or Mn^{2+}, but reactivability by divalent cations decreases with time in EDTA. ConA strongly inhibits, and α-methyl-D-mannoside or glucose (the latter much less efficiently) relieves the inhibition, indicating that the 5′-AMPase is a glycoprotein. Histidine is also inhibitory. Ouabain, phloretin, cytochalasin B, cysteine, phenylalanine, MalNEt, and IAA are without effect.

5′-AMPase activity co-distributes with pulse-bound [^{3}H]ouabain when either of two different cell-fractionation procedures are used. The 5′-AMPase activity per cell is constant at different cell densities in exponentially growing cells, and activity per unit cell volume remains constant throughout the cell cycle. These properties, together with its absence in other organelles, its stability to storage, its insensitivity to certain experimental manipulations, and its general insensitivity to inhibitors of specific transport systems make 5′-AMPase a useful quantitative marker in studies on the regulation of HeLa membrane transport systems.

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Photochemistry and Photobiology of DNA Containing 5-Iodouracil and 5-Iodocytosine

R. O. Rahn, R. S. Stafford, and C. T. Hadden

Quantum yields for the photolysis of iodinated derivatives of cytosine (leading to the loss of iodine) were measured for 313-nm radiation and were found to decrease from 0.024 for IodCMP down to 0.007 for both poly(C) and DNA containing 5-IoCyt. In DNA, approximately one clean chain break and one alkali-labile bond were formed for every ten iodines lost. Incorporation of iodinated cytosine or uracil into the DNA of *Escherichia coli* or *Bacillus subtilis* was shown to have a large effect on the biological survival following 313-nm radiation.

Effects of Platinum Antitumor Agents and Pyrimidine Dimers on the *In Vitro* Replication of T7 DNA

Neil P. Johnson,* James D. Hoeschele,† Nancy B. Kuemmerle, Warren E. Masker, and Ronald O. Rahn

The inhibition of *in vitro* T7 DNA replication by *cis*- and *trans*-dichlorodiammine platinum(II) has been measured as a function of the number of platinum moities bound to the DNA. The cis isomer inhibits replication as effectively per lesion as pyrimidine dimers, but the trans isomer is fivefold less effective.

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†Health and Safety Research Division, Oak Ridge National Laboratory.

In Vitro Packaging of Bacteriophage T7 DNA Synthesized *In Vitro*

Warren E. Masker, Nancy B. Kuemmerle, and David P. Allison

An *in vitro* DNA packaging system was used to encapsulate T7 DNA that had been synthesized by extracts prepared from gently lysed *Escherichia coli* infected with bacteriophage T7 carrying amber mutations in gene 3 or in both genes 3 and 6. Isopycnic centrifugation of density-labeled wild-type DNA was employed in an effort to separate product from template; suppressor-free indicator bacteria were used to eliminate contributions from endogenous DNA or contaminating phage. Additional controls indicated that fragmented DNA is packaged *in vitro* only with very low efficiency and that the frequency of recombination during packaging is too low to affect interpretation of these experiments. T7 DNA replicated by extracts prepared using T7 mutants deficient in both genes 3 and 6 could be packaged *in vitro* with an efficiency comparable to that found when highly purified virion T7 DNA was used. When T7 deficient in the gene 3 endonuclease but with normal levels of the gene 6 exonuclease was used, fast-sedimenting concatemer-like DNA structures were formed during *in vitro* DNA synthesis. Electron microscopy revealed that many branched and highly complex DNA structures formed during this reaction. This concatemer-like DNA was encapsulated *in vitro* with an efficiency significantly greater than that found for DNA the length of a single T7 genome.

Pyrimidine Dimer Excision in Exonuclease-Deficient Mutants of *Escherichia coli**

Warren E. Masker and John W. Chase†

The rate of pyrimidine dimer excision has been measured in *Escherichia coli* strains deficient in exonuclease V, exonuclease VII, and the 5′→3′ exonuclease of DNA polymerase I. The results suggest that a reduced

level of the 5′→3′ exonuclease of DNA polymerase I diminishes the rate of dimer excision and that an additional deficiency in exonuclease VII causes a significantly greater reduction in the cell's ability to remove dimers.

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Pyrimidine Dimer Excision in *Escherichia coli* Strains Deficient in Exonuclease V, VII, and the 5′→3′ Exonuclease of DNA Polymerase I*

John W. Chase,† Warren E. Masker, and Janet Murphy†

An isogenic series of *Escherichia coli* strains deficient in various combinations of three 5′→3′ exonucleases (exonuclease V, exonuclease VII, and the 5′→3′ exonuclease of DNA polymerase I) was constructed and examined for ability to excise pyrimidine dimers after ultraviolet irradiation. Although the *recB* and *recC* mutations (deficient in exonuclease V) proved to be incompatible with the *polAex* mutation (deficient in the 5′→3′ exonuclease of DNA polymerase I), it was possible to reduce the level of the *recB,C* exonuclease by the use of temperature-sensitive *recB270 recC271* mutants. It was found that by using strains deficient in exonuclease V postirradiation DNA degradation could be reduced and dimer excision measurements facilitated. Mutants deficient in exonuclease V were found to excise dimers at a rate comparable to wild type. Mutants deficient in exonuclease V and the 5′→3′ exonuclease of DNA polymerase I are slightly slower than wild type at removing dimers accumulated after doses in excess of 40 J/m^2. However, although strains with reduced levels of exonuclease VII excise dimers at the same rate as wild type, the addition of an exonuclease VII deficiency to a strain with reduced levels of exonuclease V and the 5′→3′ exonuclease of DNA polymerase I causes a marked decrease in the rate and extent of dimer excision. These observations support previous indications that the 5′→3′ exonuclease of DNA polymerase I is important in dimer removal and also suggest a role for exonuclease VII in the excision repair process.

*Research sponsored jointly by the Albert Einstein College of Medicine and the Division of Biomedical and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with Union Carbide Corporation.

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Workshop Summary: Excision Repair Pathways in Bacteria

Warren E. Masker

Abstract not available.

Cessation of Respiration After Far-Ultraviolet Irradiation of *Escherichia coli* B/r: Loss of Unaltered Pyridine Nucleotides to the Medium

Robert L. Schenley, Paul A. Swenson, and Jayant G. Joshi*

Cessation of respiration of *Escherichia coli* B/r cells is initiated 30 min after irradiation at 254 nm and is linked to cell death. Pyridine nucleotides begin to disappear with the onset of respiratory failure and are almost completely absent from the cells 90 min after irradiation. We studied the fate of these respiratory cofactors in a niacin-requiring mutant (RS1) grown on minimal medium containing [7-^{14}C]nicotinic acid. By 90 min after irradiation (52 J/m^2) nearly all of the acid-soluble radioactive counts appeared in the

medium. Paper chromatographic studies and a spectrophotometric assay indicated that the material was nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate. The loss of nicotinamide adenine dinucleotide was not balanced by synthesis, despite the presence of appropriate active biosynthetic enzymes for at least 90 min after UV irradiation. Analysis of the amino acid and nucleotide pools of the cells showed some loss of most of these small molecules, but the levels of a few were almost completely depleted. We conclude that the oxidized pyridine nucleotides are lost from the cell to the medium and that the loss cannot be attributed to extensive general membrane damage.

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Death of *Escherichia coli* Cells as a Consequence of Damage to DNA by Far-Ultraviolet Radiation

P. A. Swenson

This paper deals with unanswered questions concerning death of *Escherichia coli* cells after far-UV irradiation. Attention is called to poor correlations between DNA repair and survival in repair-competent cells given various pre- and post-UV treatments. Under some of these and other conditions, a large percentage of pyrimidine dimers remain unexcised. The evidence is reviewed concerning UV induction of irreversible metabolic responses associated with cell death. Since cyclic 3′,5′-adenosine monophosphate (cAMP) and its receptor protein regulate cessation of respiration and cell killing but have no known regulatory roles in DNA repair and DNA replication, death may be the result of induced biochemical and physiological responses that become irreversible.

Effects of Antipain (a Protease Inhibitor) on Respiration, Viability, and Excision of Pyrimidine Dimers in Ultraviolet-Irradiated *Escherichia coli* B/r Cells

P. A. Swenson and R. L. Schenley

The protease inhibitor antipain increases the effectiveness of UV irradiation on cessation of respiration and cell killing in *Escherichia coli* B/r cultures without affecting excision of pyrimidine dimers. The actions are similar to those caused by cyclic 3′,5′-adenosine monophosphate in irradiated cultures.

Reversibility of Nucleosome Conformation Perturbed by Urea*

M. Zama,† D. E. Olins, E. Wilkinson-Singley, and A. L. Olins

Monomer nucleosomes (ν_1) from chicken erythrocyte nuclei were diluted into 9 *M* urea plus 0.2 m*M* EDTA (pH 7.0), and urea was removed by dialysis. The ν_1 thus obtained were fractionated by sucrose gradient ultracentrifugation. Each fraction was examined in 0.2 m*M* EDTA for reversibility of ν_1 structure perturbed by urea. At least 30% of the initial amount of ν_1 exposed to urea was restored to the original structure as shown by sedimentation velocity, electron microscopy, circular dichroism, the fluorescence of ν_1 labeled with *N*-(3-pyrene) maleimide on thiol groups of H3 histone, and thermal melting.

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†Research performed while Dr. Zama was on leave from the National Institute of Radiological Sciences, Chiba-shi, Japan.

Physical Properties of Inner Histone-DNA Complexes*

P. N. Bryan,† E. B. Wright, M. H. Hsie, A. L. Olins, and D. E. Olins

Chicken erythrocyte inner histone tetramer has been complexed with several natural and synthetic DNA duplexes by salt-gradient dialysis at various protein/DNA ratios. The resulting complexes, in low-ionic-strength buffer, have been examined by electron microscopy, circular dichroism, and thermal denaturation. Electron microscopy reveals nucleosomes (ν bodies) randomly arranged along DNA fibers, including poly(dA-dT)•poly(dA-dT), poly(dI-dC)•poly(dI-dC), but not poly(dA)•poly(dT). Circular dichroism studies showed prominent histone α-helix and "suppression" of nucleic acid ellipticity ($\lambda > 240$ nm). Thermal denaturation experiments revealed Tm behavior comparable to that of H1- (or H5-) depleted chromatin. Tm III and Tm IV increased linearly with G + C% (natural DNAs) but were virtually independent of the histone/DNA ratio; therefore, the melting of nucleosomes along a DNA chain is insensitive to adjacent "spacer" DNA lengths. This suggests that Tm III and Tm IV arise from the melting of different domains of DNA associated with the core ν body.

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†Predoctoral investigator, Grant CA 09104 from the National Cancer Institute.

Nucleosome Conformation: pH and Organic Solvent Effects*

M. Zama,† D. E. Olins, B. Prescott,‡ and G. J. Thomas, Jr.‡

Monomer nucleosomes (ν_1) from chicken erythrocyte nuclei were examined in aqueous buffers ($8 > pH > 3$) and in solvent mixtures (i.e., water and ethanol, ethylene glycol, dioxane, dimethyl sulfoxide, 2-methyl-2,4-pentanediol, polyethylene glycol, sucrose, or urea). Circular dichroism, laser Raman spectroscopy of ν_1, and the fluoresence of ν_1 labeled with *N*-(3-pyrene) maleimide on thiol groups of H3 histone were employed to detect conformational transitions in ν_1. The results of the pH studies were as follows: $5.5 > pH > 4.8$, suppression of DNA ellipticity and no change of histone α-helix; $4.6 > pH > 4.2$, an irreversible increase in the B character of DNA, a slight loss of histone α-helix, and a parallel loss of pyrene excimer fluorescence; $4 > pH$, aggregation of ν_1 and protonation of the DNA bases C and A. Results obtained in the studies of ν_1 in solvent mixtures included sharp conformational transitions that variously involved an increase in the B character of DNA, a slight loss of histone α-helix, and a loss of pyrene excimer. Different solvents required different concentrations to effect these conformational changes.

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Salt Dependence of Inner Histone Tetramer-Octamer Equilibrium*

Andrew P. Butler,† Rodney E. Harrington,‡ and Donald E. Olins

The inner histone complex, extracted from chicken erythrocyte chromatin in 2 *M* NaCl at pH 7.4, has been characterized by sedimentation equilibrium and sedimentation velocity. The molecular weights determined from high-speed sedimentation equilibrium indicate that in 2 *M* NaCl the inner histones exist predominantly as a tetramer with weak association to form octamer at protein concentrations greater than 1 mg/ml. At histone concentrations below 50–100 μg/ml, substantial amounts of histone dimer are present. At higher salt concentrations, the equilibrium is shifted to favor octamer; in 4 *M* NaCl essentially all of the histone is octameric at protein concentrations greater than 200 μg/ml. Sedimentation velocity experiments

reveal a single sedimentation boundary over a wide range of salt conditions and protein concentrations, with sedimentation coefficient $s_{20,w}$ = 3.8 in 2 *M* NaCl and 4.2 in 4 *M* NaCl; this supports the concept of a rapid equilibrium between histone oligomers.

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†Postdoctoral investigator, subcontract 3322 from the Biology Division of Oak Ridge National Laboratory to the University of Tennessee.

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Nucleosomes: The Structural Quantum in Chromosomes*

Donald E. Olins and Ada L. Olins

Abstract not available.

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Electron Microscopy: A Tool for Visualizing Chromatin*

Ada L. Olins

Abstract not available.

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Interaction of B(*a*)P Diol-Epoxide with Chromatin

A. Kootstra,* T. J. Slaga, and D. E. Olins

Abstract not available.

*Postdoctoral investigator, grants CA 20076 and GM 19334 from the National Institutes of Health.

Important Hydrodynamic and Spectroscopic Techniques in the Field of Chromatin Structure*

Donald E. Olins

Combining hydrodynamic and spectroscopic techniques in the study of conformational states of ν_1 induced by a variety of perturbants has led us to a general conception: the two structural domains of ν_1 (i.e., the DNA-rich outer shell and the α-helix-rich apolar histone core) exhibit differential responsiveness. In general, the α-helical regions are more resistant than DNA conformation or ν_1 size and shape to the perturbing effects of urea, decreased ionic strength and pH, trypsin treatment, or a variety of water-miscible organic solvents. There are a number of reasonable conceptual models to explain this differential responsiveness of the structural domains of ν_1. A few of these models can be denoted with self-descriptive names – "shell-swell," "open-clam," or "unravelled." Distinction between the various models will require data from many

other biophysical techniques, such as neutron scattering studies. Whatever the exact geometry of the transitions, it is tempting to speculate that the α-helical histone core constitutes the "restoring force" of the nucleosome – returning the conformationally perturbed nucleosome to its "inactive" compact form.

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Kinetics of DNA Replication and Distribution of DNA in *Escherichia coli.* I. Theory

W. H. Olson* and M. L. Randolph†

We developed a mathematical model that determines kinetic parameters of DNA replication in an exponentially growing population of *Escherichia coli*. The DNA replication initiation rate and the time for a round of replication are given in terms of the measurable quantities: (*a*) fractional increase in DNA after inhibition of initiation and (*b*) population growth rate. The derivation comes by analogy with the queueing theory model for a system with random arrivals, constant service times, and infinitely many servers. Under reasonable assumptions about the mode of replication, we show that the distribution of the number of replication positions is Poisson and that the positions are uniformly distributed along the genome. As a by-product of the derivation, we obtain expressions for the number and weight distributions of partially replicated and full-length strands of DNA and, in particular, the number-average and weight-average molecular weights.

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Kinetics of DNA Replication and Distribution of DNA in *Escherichia coli.* II. Experimental

M. L. Randolph* and W. L. Olson†

A simultaneously developed statistical theory and essentially simple experimental measurements on a strain of *Escherichia coli* which is both thymine requiring and temperature sensitive for initiation of DNA synthesis are used to evaluate the kinetics of DNA replication and distributional characteristics of the DNA in exponentially growing bacteria. Necessary corrections to the raw experimental data and assumptions made are discussed. Under our growth conditions and assumptions, the cell doubling time is 61 min; the calculated average number of initiation positions per parent duplex DNA molecule is 0.89; the initiation rate is 0.0113 min^{-1}; the replication time is 79 min; and the number-average single-strand molecular weight is 66% of the full-strand molecular weight.

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Crystallographic Characterization of a Principal Nontoxic Lectin from Seeds of *Ricinus communis*

Chin Hsuan Wei and Chongkun Koh

Large hexagonal crystals of *Ricinus* lectin, present as a major component in the seeds of *Ricinus communis*, have been obtained at 4°C in the presence of polyethylene glycol 6000 by vapor diffusion against media containing acetic acid. The crystals are of space group P622, with hexagonal unit cell parameters $a = b =$ 166 Å and $c = 341$ Å. The asymmetric unit contains two molecules of molecular weight 125,000. The

crystals are extremely unstable, both to environmental changes and to X radiation, and have a solvent content of about 55% by volume.

Isolation of Plastid Ribosomes from *Euglena*

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Abstract not available.

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Isolation of *Euglena* Chloroplastic tRNA and Purification of Chloroplastic $tRNA^{Phe}$

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Isolation of *Neurospora* Mitochondrial Transfer RNA

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Mechanism of Suppression in *Drosophila.* VII. Correlation Between Disappearance of an Isoacceptor of Tyrosine Transfer RNA and Activation of the Vermilion Locus

K. Bruce Jacobson

The possibility that tyrosine tRNA modifies the catalytic activity of tryptophan oxygenase that is produced by the vermilion mutant *v* in *Drosophila melanogaster* is reconsidered. Dietary conditions can modify the ratio of the two major isoacceptors of tyrosine tRNA; one condition allows 85–90% to exist as the second isoacceptor, and another condition allows <5% to exist in this form. The function lacking in the vermilion mutant is partially restored when the second isoacceptor of $tRNA^{Tyr}$ is reduced to low levels (<40%), but the function is greatly reduced when this isoacceptor is present as 50% or more of the total. These data support the hypothesis that $tRNA^{Tyr}$ may be associated with and regulate tryptophan oxygenase. The corresponding isoacceptor of $tRNA^{Tyr}$ found in a suppressor mutant, *su(s)*^{2}, should not have any effect on

the function of the vermilion gene, and, indeed, it did not. The tRNAs for tyrosine, aspartic acid, and histidine all have one isoacceptor that contains nucleoside Q, and all undergo parallel changes in flies raised on the various diets. It appears that these dietary changes affect the ability to synthesize or modify Q or to remove or insert it into tRNA.

Tyrosine Transfer RNA Isoacceptors in *Drosophila:* Comparison of the *su(s)*^{2} Mutation and Wild Type

N. K. Howes* and K. Bruce Jacobson

The isoacceptors $tRNA_1^{Tyr}$ and $tRNA_2^{Tyr}$ from wild-type *Drosophila* and the isoacceptors $tRNA_A^{Tyr}$ and $tRNA_B^{Tyr}$ from the *su(s)*^{2} suppressor mutant were compared by aminoacylating these species of tRNA with ^3H- or ^{14}C-labeled tyrosine and then subjecting them to a limited T_1 RNase digestion at pH 5.8. The oligonucleotide fragments that were attached to [^{3}H]- and [^{14}C]tyrosine were mixed, resolved on RPC-5 columns, and detected by discrimination counting. Homology between $tRNA_1^{Tyr}$ and $tRNA_2^{Tyr}$ and also between $tRNA_A^{Tyr}$ and $tRNA_B^{Tyr}$ was demonstrated. No differences were found between respective tyrosyl tRNAs isolated from wild-type *Drosophila* or from the *su(s)*^{2} suppressor mutant. The findings support the hypothesis that the two isoacceptors of tyrosine tRNA in *Drosophila melanogaster* differ only by post-transcriptional modification.

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Biosynthesis of "Drosopterins" in the Head of *Drosophila melanogaster*

Dale L. Dorsett,* John J. Yim,† and K. Bruce Jacobson

Conditions are described that allow for the enzymatic conversion of dihydroneopterin triphosphate to neodrosopterin, isodrosopterin, fraction (*e*) [Schwinck and Mancini], aurodrosopterins, and sepiapterin. Synthesis of the "drosopterins" requires Mg^{2+} and either NADPH or NADH, whereas sepiapterin synthesis requires Mg^{2+} and NADPH. Drosopterin synthesis occurs enzymatically in three subcellular fractions, whereas the majority of sepiapterin synthesis occurs enzymatically in one subcellular fraction. Evidence presented suggests that sepiapterin is not a precursor to drosopterins but that they share a common precursor.

*Predoctoral investigator, Grant GM 1974 from the National Institute of General Medical Sciences.

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Biosynthesis of "Drosopterins" by an Enzyme System from *Drosophila melanogaster*

Dale Dorsett,* John J. Yim,† and K. Bruce Jacobson

Conditions are described that allow for the enzymatic conversion of dihydro-D-erythro-neopterin triphosphate to neodrosopterin, drosopterin, isodrosopterin, fraction (*e*) [Schwinck and Mancini], aurodrosopterins, and sepiapterin. Synthesis of the "drosopterins" requires Mg^{2+} and either NADPH or NADH, whereas sepiapterin synthesis requires Mg^{2+} and NADPH. Drosopterin synthesis occurs in three fractions prepared by differential centrifugation of a *Drosophila* head homogenate, while sepiapterin synthesis occurs

mainly in the 100,000*g* supernatant fraction. The evidence presented supports the hypothesis that sepiapterin is not a precursor of the drosopterins but that drosopterins and sepiapterin share a common precursor.

*Predoctoral investigator, Grant GM 1974 from the National Institute of General Medical Sciences.

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The Application of a New, Rapidly Taught Passive-Avoidance Task to the Search for a Mouse Deficient in its Capacity to Consolidate Information

William L. Byrne,* Robert C. Hermann,* Robert A. Schreiber,* J. Graves,† and K. Bruce Jacobson

The memory may be considered a process composed of various biochemical and electrical events. The capacity of mice to remember an experience for 24 hr was measured in an apparatus that allows them to touch a shiny ball and either does or does not give them a shock when they do touch. A number of inbred strains of mice were tested; five strains exhibited very good memory, one only fair, and two exhibited little or no capacity to remember the experience. This apparatus seems to have advantages over other devices and could be useful for exploring the ability of various agents to alter memory.

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Preservation of Mammalian Germ Plasm by Freezing

Peter Mazur

Although bovine sperm was first frozen successfully in 1950, more than 20 years had to elapse before the successful freezing of mammalian ova and embryos. Success came about partly through the development of a degree of understanding of the fundamentals of cryobiology. The challenge to cells during freezing is whether they survive cooling *to* low temperatures and warming therefrom. *At* low temperatures (e.g., −196°C), the absence of thermal reactions suggests that viability can be maintained a millennium or more. One critical factor in survival is the cooling rate. Too high a cooling rate prevents osmotic equilibration and results in lethal intracellular freezing. The critical cooling rate can be calculated from a physical-chemical model. For a large cell such as the mouse ovum, the critical cooling rate is about 1°C/min.

Although slow cooling is mandatory for survival, it is not sufficient. Slowly cooled cells will also be killed unless a protective additive such as glycerol or dimethyl sulfoxide is present in approximately molar concentration. These additives protect cells because during freezing they reduce electrolyte concentrations by virtue of their colligative action. A final requirement for survival is the avoidance of osmotic shock during the thawing of embryos and their subsequent return to physiologic media. Under appropriate conditions survivals after freezing approach 90% on the basis of both cleavage *in vitro* and development to term in foster mothers. Thus, low-temperature storage can be used in the preservation of mutant strains. It should also prove to be a valuable tool for eliminating or assessing genetic drift. To date, preimplantation embryos of mice, rats, rabbits, sheep, goats, and cattle have been frozen successfully.

Biological Implications of the Viking Mission to Mars

Peter Mazur, Elso S. Barghoorn,* Harlyn O. Halvorson,† Thomas H. Jukes,‡
Isaac R. Kaplan,§ and Lynn Margulis‖

A central purpose of Viking was to search for evidence that life exists on Mars or may have existed in the past. The mission carried three biology experiments, the prime purpose of which was to seek for existing microbial life. In addition, the results of a number of the other experiments have biological implications: (*a*) The elemental analyses of the atmosphere and the regolith showed or implied that the elements generally considered essential to terrestrial biology are present. (*b*) Unexpectedly, no organic compounds were detected in Martian samples by an instrument that easily detected organic materials in the most barren of terrestrial soils. (*c*) Viking obtained direct evidence for the presence of water vapor and water ice, and it obtained strong inferential evidence for the existence of large amounts of subsurface permafrost now and in the Martian past. However, it obtained no evidence for the current existence of liquid water possessing the high chemical potential required for at least terrestrial life, a result that is consistent with the known pressure-temperature relations on the planet's surface. (Liquid water is believed to be an absolute requisite for life.) On the other hand, the mission did obtain strong indications from both atmospheric analyses and orbital photographs that large quantities of liquid water flowed episodically on the Martian surface 0.5 to 2.5 billion years ago.

The three biology experiments produced clear evidence of chemical reactivity in soil samples, but it is becoming increasingly clear that the chemical reactions were nonbiological in origin. The unexpected release of oxygen by soil moistened with water vapor in the gas exchange (GEX) experiment together with the negative findings of the organic analysis experiment lead to the conclusion that the surface contains powerful oxidants. This conclusion is consistent with models of the atmosphere. The oxidants appear also to have been responsible for the decarboxylation of the organic nutrients that were introduced in the label release (LR) experiment. The major results of the GEX and LR experiments have been simulated at least qualitatively on Earth. The third experiment, pyrolytic release, obtained evidence for organic synthesis by soil samples. Although the mechanism of the synthesis is obscure, the thermal stability of the reaction makes a biological explanation most unlikely. Furthermore, the response of soil samples in all three experiments to the addition of water is not consistent with a biological interpretation.

The conditions now known to exist at and below the Martian surface are such that no known terrestrial organism could grow and function. Although the evidence does not absolutely rule out the existence of favorable oases, it renders their existence extremely unlikely. The limiting conditions for the functioning of terrestrial organisms are not the limits for conceivable life elsewhere, and accordingly one cannot exclude the possibility that indigenous life forms may currently exist somewhere on Mars or may have existed sometime in the past. Nevertheless, the available information about the present Martian environment puts severe constraints and presents formidable challenges to any putative Martian organisms. The Martian environment in the past, on the other hand, appears to have been considerably less hostile biologically, and it might possibly have permitted the origin and transient establishment of a biota.

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Gene 24–Controlled Osmotic Shock Resistance in Bacteriophage T4: Probable Multiple Gene Functions

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By mixed infections with conditional lethal mutations in the head genes and an osmotic shock-resistant mutant, we have demonstrated that osmotic shock resistance is controlled by gene 24. By acrylamide gel electrophoresis combined with the "immune replicate" technique, we confirmed the positions of gene products 24 and 24*; gp24 *ts* showed a significantly slower mobility. Both osmotic shock-resistant and -sensitive mature phages contain gp24*. Giants constructed with the Os^R phage showed the same surface lattice as normal phage.

Through temperature shift experiments with 24 (*ts*L90) alone and in combinations, we studied the phages which are matured after shift to permissive temperature in the absence of new protein synthesis. These results strongly suggest that only a fraction of the total phage complement of gene 24–controlled proteins is involved in determining the phenotype of shock resistance, while the remainder is necessary to mature the head.

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Water Movement: The Critical Factor for the Preservation of Mouse Embryos

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CANCER AND TOXICOLOGY SECTION

Few diseases have occupied the minds of the public, the press, and the politician as dominantly as cancer. Not only is there a fear of the disease, but an apprehension, whether justified or not, that the potential effects of the products and effluents of the technology that has provided our affluence hangs like the Sword of Damocles. The long period between exposure to a carcinogenic agent and the ultimate disease increases the apprehension and confounds a rational approach. Thus, cancer research has a great responsibility, and the researchers have a problem as complex as biology itself. The complexity of cancer, its causes, the mechanisms of its causation, and its prevention present an exciting challenge that demands multidisciplinary but interrelated approaches.

The research of the Section includes studies that span every level of organization from molecular to whole animal. The studies reflect the gamut of agents that are implicated in cancer – from sunlight to ionizing radiation, from simple and common chemicals to the more complex compounds which undergo tissue and species-specific enzymatic metabolism, and from fibers to the more animate and ubiquitous viruses. The interaction of carcinogenic agents with macromolecules, in particular DNA, appears to be fundamental to the initiation of the events leading to cancer, and therefore studies on transport, uptake, binding, metabolic activation, and degradation are a prominent feature. The sequelae of exposure to carcinogenic agents are varied, but in normal cells repair of damage, especially to DNA, is a common consequence; therefore, an understanding of the role of repair in the prevention of cancer-producing events has long been an important area of research in the Section and in the Division. More recently the realization of the possibility that errors leading to mutation can be introduced by certain forms of repair processes has strengthened the need for an even better understanding of repair. The study of DNA repair bridges the work of all three sections. The interrelationship of many other varied studies in the three sections underlines the danger of dividing research into basic and applied. The repair of DNA damage was initially studied in microbial systems; now such studies are being carried out with normal human cells and human cells with specific DNA repair defects. Repair of ultraviolet and ionizing radiation damage was the major focus of much of this work, but recently in this laboratory the repair of damage induced by chemical carcinogens, for example the polycyclic aromatic hydrocarbons, has been characterized. It has been found that the damage induced by the active diol-epoxide metabolites of benzo [*a*] pyrene is repaired by the same excision repair system as ultraviolet radiation-induced damage.

The dissection of the complex metabolic pathways for various chemical carcinogens continues to be carried out in various ways. These studies are essential for an understanding of (*a*) the important changes in DNA induced by mutagens and carcinogens, (*b*) the organotropism of many carcinogens, and (*c*) the species specificity of mutagens and carcinogens.

Recent studies here have established that a diol-epoxide is probably the major metabolite responsible for the mutagenic properties, not only of benzo [*a*] pyrene but also 7,12-dimethylbenz [*a*] anthracene (DMBA). This result is of particular importance to an understanding of mechanisms common to different carcinogens of the polycyclic aromatic hydrocarbon type. The identification of the important metabolites makes it possible to investigate the relevant DNA adducts and their repair, and both these lines of research are being pursued actively. The development of methods of detection of the induced changes in the DNA, the membranes, and the antigenic properties is being pursued. Solid state radioimmunoassays for tumor-specific proteins is one example.

The ability both to assess accurately possible mutagens and carcinogens and to investigate mechanisms depends on appropriate test and model systems. In the case of mutagenesis, the development of reliable mammalian systems has been accomplished. Furthermore, the problem of obtaining appropriate metabolism of chemicals in order to test the potential mutagenic or carcinogenic effects has been largely solved with the development of cell-mediated assays. These assays, using the appropriate cells for metabolism of potential mutagens and carcinogens, allow precise assessment of chemicals for their mutagenicity and also the investigation of organ and species specificity. Carcinomas that stem from epithelial cells are the common forms of cancer. However, most of the *in vitro* cell transformation studies have been carried out on fibroblast cell systems. Recently a major effort has resulted in some very useful *in vitro* respiratory epithelial systems which, combined with tracheal grafts, have made it possible to probe systematically the process of neoplasia. A method of recolonization of tracheas with cells which can be treated *in vitro* has also been developed recently. Similar development of skin epithelial *in vitro* systems is also under way. The development of *in vitro* cell systems is essential for the study of the mechanisms of mutagenesis and carciongenesis, but the development of good *in vivo* systems is of equal importance. In this laboratory two groups have developed very sophisticated bioassay systems using mouse skin as the test system. In one, the concentration has been on the determination of both initiating and promoting capability of compounds. Adjunct experiments are concerned with the different mechanisms involved in the two major stages of carcinogenesis. In another approach, methods have been developed for obtaining dose-response relationships, especially for reference carcinogens, in representative strains that differ in their susceptibility.

A more optimistic area of cancer research has recently opened up – namely, chemoprotection. Many chemical compounds have the property of reducing the carcinogenic potential of various compounds. The mechanisms of the protection vary and are under study.

Currently the data that have been accumulating from the large-scale radiation toxicity experiments are being analyzed, and the conclusions are being used by advisory bodies to assess the risk of both low doses of irradiation and irradiation at low-dose rates. The insights gained from these experiments have been the basis of current experiments that are more concerned with the mechanisms of radiation carcinogenesis.

Virological research, a major feature of the Section's program for so many years, continues to provide interesting results. The introduction of DNA transfection techniques has provided an exciting method to elucidate the genetic interactions between host cells and RNA tumor viruses. The role of transfer RNA molecules as primers for the initiation of reverse transcription in the life cycle of RNA tumor viruses is now being investigated. An experimental approach has been developed to detect retrovirus-related cellular messenger RNAs that contain "leader" sequences with primer transfer RNA binding properties. In this way it is possible to look for altered genetic expression in human cancers. Also the transfer RNA in 13 different tumor viruses has been characterized. Synthesis of a specific type C viral gene product has been induced and quantified in fetal baboon fibroblasts. This gene product was differentially expressed, which may explain the failure to detect complete infectious endogenous retroviruses from tissues of humans and other primate species.

This varied research is now supported by many agencies, including U.S. Department of Energy, National Cancer Institute, National Institute of Environmental Health Sciences, National Aeronautical and Space Administration, and the Environmental Protection Agency. Clearly, the Division has become a national resource capable of responding to current national needs. In order to increase the breadth of our capability, a new group – namely, Toxicology – has been added. This new program is designed to study mechanisms in such a way that both testing of potentially toxic agents and the assessment of the risk they pose can be approached in a rational and more productive manner. A considerable effort is placed on heavy metal toxicity. Studies are also being carried out on the pathogenesis of lung fibrosis, especially in the case of exposure to agents that interact in rather unpredictable ways. These studies are important for the understanding of the etiology of chronic lung disease.

Lysine Transfer RNA Is the Predominant Transfer RNA in Murine Mammary Tumor Virus

Larry C. Waters

The method of aminoacylation and subsequent identification of the esterified amino acids was used to characterize the transfer RNAs in murine mammary tumor virus. Lysine tRNA was the major tRNA in both "free" 4S RNA and "70S-associated" 4S RNA in virus derived from either tissue culture or mouse milk.

Temporal Advancement of Diethylnitrosamine Carcinogenesis in Aging Mice

Neal K. Clapp, Eugene H. Perkins, William C. Klima, and Lucia H. Cacheiro

Female BALB/c mice were given diethylnitrosamine in their drinking water beginning at 2.5, 9.5, and 17 months of age (cumulative dose about 300–400 mg/kg body weight) or were untreated. Median times of death differed significantly ($P < 0.01$); they were 193, 168, and 125 days, respectively, after cessation of diethylnitrosamine treatment. Induced tumors in the three respective age groups were of squamous forestomach (88, 87, and 84%), vascular tumors of liver (11, 13, and 16%), and adenomas of the lung (65, 56, and 54%). Controls had no forestomach or liver tumors and relatively low incidences of lung tumors. The fact that aging mice have similar incidences and types of tumors of the same size and in the same tissues, but at an earlier time, shows that aging mice can metabolize diethylnitrosamine, and the most significant alteration in the tumor process is temporal advancement (the older the mice were when given diethylnitrosamine, the earlier they developed tumors). This observation may be related, in part, to an identified age-dependent decrease in immunocompetency or to other age-related changes (e.g., vascular or hormonal), which could explain temporal advancement in the tumorigenic process.

Studies on Nitrosamine Metabolism. III. Lipid-Associated Radioactivity After [^{14}C] Dimethylnitrosamine and Effect of Dimethylnitrosamine on ^{32}P Incorporation into Phospholipids

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Abstract not available.

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Analysis of the Electrophoretic Pattern of Nonspecific Esterases of Black Bear Serum

J. Patrick Daugherty,* Diane K. Beeman,† and Michael R. Pelton†

The serum of 43 black bears (*Ursus americanus*) from the Great Smoky Mountains National Park was analyzed for nonspecific esterases by use of concave gradient polyacrylamide gel electrophoresis. The sera were resolved into approximately 11 bands of enzyme activity. Certain esterase bands are quantitatively different in males and females, and alterations in the electrophoretic profile may be correlated with the physiological state and previous history of the animals.

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Sexual Maturation in Two Strains of Mice

K. A. Davidson, J. M. Holland, L. C. Gipson, and J. W. Hall

Several parameters were used to study sexual maturation in C3Hf/Bd (C3) and C57BL/6fBd (B6) female mice. These include somatic weight, uterine weight, ovarian weight, age at vaginal opening, age at first litter, and plasma estradiol levels. A strain difference was observed for each parameter, with the exception of age at first litter. The greatest difference between the two strains, indicating that C3 females mature earlier than B6 females, was in the age at vaginal opening: C3 females were 28.4 ± 0.7 days old at the time of vaginal opening, whereas B6 females were 43.1 ± 0.42 days old. The difference between these two strains appears to be genetic, as shown by the fact that vaginal opening occurred in $C3BF_1$ and $BC3F_1$ hybrids of these strains at 32.1 ± 0.21 and 33.4 ± 0.43 days respectively. Maternal factors had very little influence on the age of sexual maturation; foster-nursing C3 litters on B6 females and vice versa did not appreciably alter the parameters that were measured.

Survival and Cause of Death in Aging, Germfree Athymic Nude and Normal Inbred C3Hf/He Mice

J. M. Holland, T. J. Mitchell,* L. C. Gipson, and M. S. Whitaker

Life span and relative risk for specific diseases were compared between male and female athymic and normal C3Hf/He mice under germfree conditions. Athymic mice showed significantly reduced overall survival rates, most of which could be attributed to an increased risk for lymphoreticular neoplasms. Although athymic mice were at an increased risk for lymphoreticular diseases, they were at significantly reduced risk for some, but not all, groups of solid tumors. A decreased probability of death with or from solid tumors was a possible explanation for the observation of a significantly greater proportion of athymic mice dying from "natural causes." The fact that maximum longevities attained by athymic mice were no different than longevities of normal controls suggests that thymic aplasia does not influence life span per se but does influence the relative incidence of specific pathological states. A specific consideration was whether chronic degenerative diseases indicative of autoimmune processes (vasculature, renal glomerulus, or thyroid) were more prevalent in aging athymic mice. Histologic evidence for these progressive disease processes was not obtained; thus it appears unlikely that clinically significant autoimmune phenomena are significant factors controlling morbidity or mortality in germfree athymic mice on a C3Hf/He background.

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Survival and Disease Risk in X-Irradiated, Germfree Athymic Nude and Normal C3H Mice

J. Michael Holland

C3Hf/He athymic and normal inbred mice were irradiated (300 rads of X rays, acute, whole body) at 10 weeks of age and maintained for life under germfree conditions. The mortality rate was 2.2 times the control rate in athymic female mice and 2.0 times the control rate in athymic male mice. The single most important cause of excess mortality was lymphoreticular tissue neoplasms. The radiation exposure caused significant life shortening in the female but not in the male athymic mice. The life-shortening effect was significantly greater in both female and male control (euthymic) mice than in the athymic groups. Age-adjusted relative risk for various lethal and nonlethal solid tumors appeared to be reduced in athymic compared with euthymic mice.

Carcinogenicity of Syncrudes Relative to Natural Petroleum as Assessed by Repetitive Mouse Skin Application

J. M. Holland, M. S. Whitaker, and J. W. Wesley

The relative carcinogenicities of coal- and shale-derived liquid crude were compared with a composite blend of natural petroleum using discontinuous exposure of mouse skin. All of the syncrudes were carcinogenic while the natural crude composite was negative following three-times-weekly application of 50% w/v solutions for 22 weeks, followed by a 22-week observation period. In addition to eliciting progressive squamous carcinomas, the syncrudes were also capable of inducing persistent ulcerative dermatitis. This inflammatory or necrotizing potential appeared to be inversely proportional to the carcinogenicity of the material. A measure of the relative solubility of the materials in mouse skin was obtained by quantitation of native fluorescence in frozen sections of skin. There appeared to be a general, although nonquantitative, association between fluorescence intensity in sebaceous glands and carcinogenicity in epidermal cells; however, it will be necessary to examine a greater number of samples to establish such a correlation.

The feasibility of using athymic mice to compare the relative *in vivo* susceptibility of intact human and mouse skin to carcinogenic hydrocarbons has been evaluated. Although the approach is technically feasible, the small proportion of grafts that survive indefinitely are evidence that many technical improvements will be necessary before this approach can be exploited.

Correlation of Fluorescence Intensity and Carcinogenic Potency of Synthetic and Natural Petroleums in Mouse Skin

J. M. Holland, M. S. Whitaker, and J. W. Wesley

After standardized topical exposure of mouse skin to synthetic and natural petroleums, the penetration and persistence of the fluorescent components have been quantitated. Carcinogenicity of the same materials was determined by short-term continuous skin application followed by a period without treatment to allow expression of malignantly transformed epidermal cells. The observations suggest that degree of fluorescence at 24 hr, persistence of the fluorescence, and absence of direct cytotoxicity are positively correlated with carcinogenicity in mouse skin.

Determination of Causes of Death in Animal Survival Experiments

J. M. Holland

Abstract not available.

Life Shortening in RFM and BALB/c Mice as a Function of Radiation Quality, Dose, and Dose Rate

John B. Storer, Louis J. Serrano, Edgar B. Darden, Jr.,* Mark C. Jernigan, and Robert L. Ullrich

A population of nearly 30,000 RFM and 11,000 BALB/c mice were exposed to graded radiation doses of gamma rays at a high or intermediate dose rate, or to fission neutrons at a high or low dose rate. Effects on life shortening are reported. The dose-response curve for gamma rays in RFM female mice at the high rate showed multiple changes in slope. In the region of 0–50 rads, however, life shortening was proportional to the square of the dose. For RFM male mice after irradiation at the high dose rate, and for both RFM and BALB/c female mice irradiated at the intermediate dose rate, the curve was linear over the entire range of doses tested. Linear dose-response relationships were also observed with neutrons at both dose rates over the lower end of the dose range. The relative biological effectiveness (RBE) of neutrons in the 0- to 50-rad range for RFM female mice irradiated at high dose rates was proportional to the -0.5 power of neutron dose; that is, the RBE increased with decreasing total dose.

Gamma rays were less effective in shortening life when delivered at an intermediate dose rate than at a high dose rate. With neutrons, the low dose rate was more effective than the high dose rate at high total doses for both strains. For the RFM, the low-dose-rate neutron irradiation at low total doses was less effective than the high dose rate, while no dose-rate effect was observed for BALB/c mice in the low dose range.

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Induction of Lung Tumors in RFM Mice After Localized Exposures to X Rays or Neutrons

R. L. Ullrich, M. C. Jernigan, and L. M. Adams

At present, studies on dose-response relationships for tumor induction after exposure to fission neutrons and X or gamma rays have supplied only limited data sufficient to allow analysis of the relation between neutron dose and relative biological effectiveness. The purpose of the present study was to compare the dose-response relationships for the induction of lung tumors in RFM mice after localized thoracic exposures to X rays or fission neutrons. Animals were killed 9 months after localized irradiation, their lungs were cleared, and the tumors were counted to determine both percentage of incidence and tumors per mouse. From these data, the relationship between tumors per mouse and X-ray dose could be described adequately by a linear quadratic model ($P > 0.90$), and linearity could be rejected over the dose range 0–900 rads. For neutrons the relationship between tumors per mouse and dose over the range 0–25 rads could be described by a number of relationships, including a linear, a dose-squared, or a linear dose-squared model. For any of these relationships, the relative biological effectiveness increased with decreasing neutron dose from 25 at 25 rads to 40 at 10 rads.

The Influence of Dose, Dose Rate, and Radiation Quality on Radiation Carcinogenesis and Life Shortening in RFM and BALB/c Mice

R. L. Ullrich and J. B. Storer

Over the past several years we have conducted large-scale experiments in mice on the late biological effects as a function of dose, dose rate, and radiation quality. Specifically, we have studied the effects produced by ^{137}Cs gamma rays delivered at a high (45 rads/min) or intermediate (8.2 rads/day) dose rate and the effect of fission neutrons at a high (25 rads/min) and low (1 rad/day) rate in a population of nearly 30,000 RFM and 11,000 BALB/c mice. Gamma ray doses ranged from 10–400 rads with the RFM and from 50–400 rads with the BALB/c, while neutron doses ranged from 5–200 rads with both strains. Data from these studies are now available both for life shortening and for the induction of a variety of neoplastic diseases. The present paper will present an overview of these data and the general findings, and subsequent publications will present a detailed analysis of each aspect. A variety of neoplasms were sensitive to induction after radiation exposure, including tumors of both reticular tissue origin (leukemia, lymphoma, etc.) and solid tumors. For the RFM, thymic lymphomas were the dominant reticular tissue neoplasm, while the majority of solid tumors were either lung adenomas or fit into the broad category of endocrine-related tumors, including ovarian, pituitary, harderian, and uterine tumors. The BALB/c was much less sensitive to induction of reticular tissue neoplasms. The tumors that were most sensitive to induction included malignant lung carcinomas, mammary adenocarcinomas, and ovarian tumors.

In general for both life shortening and tumor induction after gamma ray exposures, when the low to intermediate dose range was sufficiently defined, linearity could be rejected, and a dose-squared or linear dose-squared relationship adequately fit the data. For neutron exposures, on the other hand, linear relationships were the general finding. The relative biological effectiveness for neutrons varied with tumor type and total dose level. For gamma ray irradiation, the intermediate dose rate resulted in a decreased effectiveness in all cases, whereas for neutron exposures the dose-rate relationships were more complex.

Interactions of Radiation and Chemical Carcinogens

R. L. Ullrich

Abstract not available.

A Stable Association Between Pulse-Labeled Nuclear RNA and Chromatin: hnRNP Particles Are Components of Chromatin

Michael C. MacLeod* and Ziva Reuveny†

In a line of AKR mouse embryo fibroblasts which produces murine leukemia virus, about 90% of the nuclear RNA that becomes labeled in a 30-min pulse with [^{3}H]uridine is associated with chromatin. This association in minimally sheared chromatin is maintained as the ionic strength is raised to 0.4 *M* NaCl; above this concentration chromatin and RNA are solubilized concomitantly by increasing salt concentrations. When the chromatin is solubilized by sonication (DNA length about 1 kilobase), the RNA remains associated, as evidenced by its insolubility in low concentrations of $MgCl_2$ and in NaCl solutions; it is demonstrated that this insolubility is not a property of nuclear ribonucleoprotein (RNP) particles. These results are extended to a number of cell types, to cells labeled in the presence and absence of actinomycin D, and to chromatin prepared under conditions expected to yield hnRNP particles. Possible roles for this chromatin-associated RNA in messenger RNA biosynthesis and chromatin structure are discussed.

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Chemical Carcinogenesis*

James K. Selkirk

This chapter on chemical carcinogenesis summarizes the current state of the art in the elucidation of the mechanism of action, target tissue site, and ultimate metabolic fate of the carcinogen for several environmentally prevalent chemicals. The major areas discussed are polycyclic aromatic hydrocarbons, aliphatic nitrosamines and nitrosamides, aromatic amines, and aflatoxins. All of the carcinogens are in some degree activated or processed by mixed functions oxidases predominantly found in liver. The generally accepted hypothesis that all studied chemical carcinogens are electrophilic in nature and tend to seek out and alkylate nucleophilic sites is discussed for each of the compounds mentioned.

The chapter is written in textbook style with key referencing so the reader can locate salient and critical papers on each of the compounds discussed.

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Metabolism of Benzo[*a*]pyrene: Comparison of Rodent Liver and Embryonic Cells in Tissue Culture*

James K. Selkirk and Michael C. MacLeod

Abstract not available.

*Research sponsored jointly by the National Cancer Institute under contract Y01-CP-50200, and the Division of Biomedical and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with Union Carbide Corporation.

Comparative Metabolism of Benzo[*a*]pyrene in Rodent Liver and Embryonic Cells in Tissue Culture*

James K. Selkirk and Friedrich J. Wiebel†

High-pressure liquid chromatography has been utilized to separate and compare benzo[*a*]pyrene activation and detoxification products between rat, mouse, and hamster hepatic microsomes and mouse and hamster embryo cell cultures. Although metabolite profiles exhibited the same type of derivatives, marked quantitative variation was observed. Microsomal preparation produced large amounts of noncarcinogenic phenols, whereas intact cell metabolism favored diol formation. These results are in agreement with reactivation of metabolic diols as substrates for further activation to a more proximate carcinogenic species of benzo[*a*]pyrene and cautions against extrapolating metabolic results from any single test system to other species or tissues.

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Nucleoside Excretion and Transfer RNA Turnover in Normal and Thymic-Lymphoma-Bearing C57BL/6 Mice

Mayo Uziel and L. H. Smith

The tRNA composition, including the modified nucleosides, and the urinary excretion of modified nucleosides were compared in normal C57BL/6 mice, in those with X ray–induced thymic lymphoma, and in those bearing Krebs 2 ascites tumors. Of the 26 modified nucleosides observed in thymocyte tRNA, 15 were excreted by normal or tumor-bearing animals; these include tc^6A, ψ, U, m^1I, ac^4C, m^1G, G, m_2^2G, A, m^7G, m^1A, and m^3C. Four additional unidentified compounds were excreted which have properties of nucleosides. ψ was excreted at a rate of 450 ± 95 nmol/ml urine. The precision of the analyses is increased by normalizing the data to the urine creatinine concentration. The rate becomes 560 ± 56 nmol/mg Cr. The results showed no simple relationship between tumor tRNA composition and the increased nucleoside excretions. The levels of modification of individual nucleosides in tumor tRNA were either the same as or less than those in the normal thymocyte tRNA. Moderate fasting studies showed up to twofold increases in excretion of the modified nucleosides.

Pyrimidine Nucleoside, Pseudouridine, and Modified Nucleoside Excretion by Growing and Resting Fibroblasts

Mayo Uziel and J. K. Selkirk

The excretion of pyrimidine nucleoside from hamster embryo fibroblasts in culture has been found to be dependent on the growth stage of the cells with the greatest accumulation occurring during cell quiescence. The major nucleoside excretion products, uridine and cytidine, have been found to be both normal end products of RNA metabolism and the major nucleoside excretion products from cultured cells. The modified nucleosides *N*-1-methylguanosine, *N*-2-methylguanosine, *N*-2-dimethylguanosine, *N*-6-dimethyladenosine, *N*-4-acetylcytidine, *N*-1-methylinosine, pseudouridine, and 5-methylcytidine have also been found, as well as several unidentified nucleosides. This technique will be used to compare and contrast the RNA metabolism in normal and malignantly transformed cells.

Excretion of Nucleosides: Isolation of 3-Methylcytidine and N^4-Acetylcytidine from Normal Human Urine

Mayo Uziel and Stanton A. Taylor*

The modified ribonucleosides in normal human urine have been concentrated by affinity chromatography by use of an immobilized phenylboronate. Two additional nucleosides, 3-methylcytidine and N^4-acetylcytidine, have been identified by several liquid chromatography systems, ultraviolet spectra, identification of degradation products, and comparison to authentic samples. The nucleosides are excreted at rates of 0.7 ± 0.4 mg/day for N^4-acetylcytidine and 0.6 ± 0.4 mg/day for 3-methylcytidine. We have observed isosbestic points at 279 nm and 333 nm between pH 9 and pH 1 for N^4-acetylcytidine; the half-life for this compound is 16 hr at pH 8.8 and 23°C. There are 15 additional ultraviolet-absorbing substances present in minor amounts on the chromatogram that have not been identified.

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Cell Specificity in Metabolic Activation of the Potent Carcinogens Aflatoxin B_1 and Benzo[*a*]pyrene to Mutagens for Mammalian Cells*

Robert Langenbach,† Heather J. Freed,† Dina Raveh,‡ and Eliezer Huberman

Induction of ouabain-resistant mutants by the carcinogens aflatoxin B_1 (AF) and benzo[*a*]pyrene (BP) could not be determined in Chinese hamster V79 cells, which do not metabolize these carcinogens. Mutagenesis with AF was obtained after co-cultivating the V79 cells with rat hepatocytes (hepatocyte-mediated assay) which can metabolize this carcinogen, and mutagenesis with BP was obtained after co-cultivation with fibroblasts (fibroblast-mediated assay) which can metabolize polycyclic aromatic hydrocarbons. AF, which is not metabolized by fibroblasts, was also not mutagenic in their presence. In the hepatocyte-mediated assay, BP was not mutagenic, although on a per-cell basis the liver cells metabolized this carcinogen 4–5 times as efficiently as fibroblasts. The mutagenic activity of BP in the fibroblast-mediated assay but not in the hepatocyte-mediated assay and the inverse situation with AF are in agreement with the *in vivo* activities of these two carcinogens.

These results indicate that a cell specificity in chemical carcinogenesis can be investigated by the cell-mediated mutagenesis assay. Furthermore, while the metabolism of procarcinogens is required to manifest mutagenic activity, overall metabolism alone is not sufficient to predict the activity of a carcinogen in a given cell type.

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Cell-Mediated Mutagenesis and Cell Transformation of Mammalian Cells by Chemical Carcinogens*

Eliezer Huberman and Robert Langenbach

Naturally occurring chemical carcinogens are usually chemically nonreactive and have to be metabolically activated in order to exert the biological effects including mutagenicity. Many mammalian cell lines – including Chinese hamster V79 cells, which are suitable for studies on mutagenesis – are not able to activate these chemicals metabolically. Mutagenesis of these cells by carcinogenic polycyclic hydrocarbons, nitrosamines, and aflatoxins can be achieved when tested in the presence of normal fibroblasts or hepatocytes which are able to metabolize these carcinogens. Furthermore, our studies indicated that there is a relationship between the degree of mutant induction and the degree of carcinogenicity of the different chemicals tested. By simultaneously measuring the frequency of cell transformation and the frequency of mutation at one locus (ouabain resistance) in the same cell system, it was possible to estimate the genetic target site for cell transformation. The results indicated that the target site for transformation is approximately 20 times larger than that determined for ouabain resistance. The results suggest that cell transformation may be due to a mutational event and the mutation can occur in one out of a small number of the same or different genes, and that the cell-mediated mutagenesis approach may be a valuable means of detecting tissue-specific carcinogens.

*Research sponsored jointly by the National Cancer Institute under Interagency Agreement 40-636-77 and the Division of Biomedical and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with Union Carbide Corporation.

Cell-Mediated Mutagenesis by Chemical Carcinogens*

Eliezer Huberman and Robert Langenbach

The cell-mediated mutation system with Chinese hamster V79 cells as the target organism and with the proper choice of metabolizing cells can be useful in detecting mutagenic activities of different classes of chemical carcinogens. When fibroblastic cells were used as the metabolizing cells, a correlation between the *in vivo* carcinogenic activity and the *in vitro* mutagenic activity of eleven polycyclic aromatic hydrocarbons was observed. When primary liver cells were used as the metabolizing cells, three known liver carcinogens were demonstrated to be mutagenic by the cell-mediated assay, while two noncarcinogenic analogues were nonmutagenic. These results from the cell-mediated system suggest that the reactive intermediates of the carcinogens are stable enough to be transferred from the metabolizing cells to the V79 cells. The cell-mediated mutagenesis system is a simple *in vitro* assay that may simulate the *in vivo* situation. This approach could be extended to the co-cultivation of cells from other organs or tissues with mutable mammalian cells.

*Research sponsored jointly by the National Cancer Institute under Interagency Agreement 40-636-77 and the Division of Biomedical and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with Union Carbide Corporation.

Mutagenicity and Tumor-Initiating Activity of Fluorinated 7,12-Dimethylbenz[*a*]anthracenes*

Eliezer Huberman and Thomas J. Slaga

7,12-Dimethylbenz[*a*]anthracene (DMBA) and its 1-, 2-, 5-, and 11-fluoro derivatives were tested to determine their mutagenicity for ouabain resistance in Chinese hamster V79 cells and their tumor-initiating activity in mouse skin. Because V79 cells do not metabolize polycyclic aromatic hydrocarbons, mutagenesis was tested both in the presence and in the absence of golden hamster embryo cells capable of metabolizing polycyclic aromatic hydrocarbons. Neither DMBA nor any of the fluorinated derivatives showed mutagenicity for V79 cells in the absence of the golden hamster cells. In the presence of these cells, DMBA and

11-fluoro-DMBA exhibited a comparable mutagenic response that was dose dependent. At a dose as low as 0.01 μM, a seven- to tenfold increase in the frequency of ouabain-resistant mutants was observed. The other derivatives were either inactive or required more than a 1000-fold higher dose to induce a comparable mutagenic response. Similarily, both DMBA and 11-fluoro-DMBA at 100 nmol initiated tumors in 100% of the tested mice, whereas the other fluorinated derivatives at this dose initiated skin tumors in 15% or less of the treated mice. The average number of papillomas per mouse induced by 1-, 2-, and 5-fluoro-DMBA was more than 100-fold lower than the number induced by DMBA or 11-fluoro-DMBA. These results suggest that carbon positions 1 and 2 of DMBA, which are located at the "bay region," and position 5, which is located at the "K-region," are involved in the metabolic activation of DMBA into mutagenic and carcinogenic metabolites.

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Identification of 7,12-Dimethylbenz[*a*]anthracene Metabolites that Lead to Mutagenesis in Mammalian Cells*

Eliezer Huberman, Ming W. Chou,† and Shen K. Yang†

The mutagenicity of 7,12-dimethylbenz[*a*]anthracene (DMBA) and 13 of its enzymatically derived metabolites was tested with Chinese hamster V79 cells for identification of the mutagenic metabolites of DMBA. The metabolites consisted of 7-hydroxymethyl-12-methylbenz[*a*]anthracene, 7-methyl-12-hydroxymethylbenz[*a*]anthracene, 7,12-dihydroxymethylbenz[*a*]anthracene, 7-formyl-12-methylbenz[*a*]anthracene, 7-methyl-12-formylbenz[*a*]anthracene, three *trans*-3,4-diols, two *trans*-5,6-diols, and three *trans*-8,9-diols, all of which derived from DMBA or from the methyl-hydroxylated derivatives. Mutations were characterized by resistance to ouabain and 6-thioguanine. None of the tested metabolites were mutagenic in V79 cells, which do not metabolize polycyclic aromatic hydrocarbons; therefore, mutagenesis in the V79 cells was tested in the presence of golden hamster cells capable of metabolizing polycyclic aromatic hydrocarbons (cell-mediated assay). In this assay DMBA, 7-hydroxymethyl-12-methylbenz[*a*]anthracene, 7-methyl-12-hydroxymethylbenz[*a*]anthracene, and their *trans*-3,4-diols were mutagenic for both genetic markers, and the mutagenic response increased as a function of the hydrocarbon dose. All other metabolites were either inactive or showed up to a fourfold higher mutation frequency than the untreated V79 cells for ouabain and 6-thioguanine resistance. The DMBA-*trans*-3,4-diol was the only metabolite that was more active than DMBA itself; at an equal concentration of 0.05 μM it was six to eight times more active than DMBA in inducing both ouabain and 6-thioguanine resistance. Mutagenesis with this diol was determined at a dose as low as 0.01 μM. Mutagenesis by DMBA and the *trans*-3,4-diols was inhibited by 7,8-benzoflavone, an inhibitor of mixed-function oxidases. Analysis of DMBA metabolism in intact golden hamster cells indicated that DMBA-*trans*-3,4-diol is one of the major metabolites produced. Our results, therefore, suggest that DMBA-*trans*-3,4-diol may be metabolized to a diol-epoxide, presumably the *trans*-3,4-diol-1,2-epoxide, which may be a major reactive metabolite responsible for DMBA mutagenicity in mammalian cells.

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γ-Glutamyl Transpeptidase and Malignant Transformation of Cultured Liver Cells*

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T. D. Pugh,‡ and S. Goldfarb‡

The relationship between γ-glutamyl transpeptidase (GGT) and malignant cell transformation was analyzed in malignant and nonmalignant cultured epithelial cell lines derived from rat livers and fibroblastic cell types derived from hamsters and mice. GGT activity was prominent (25–90% of cells) in three out of five malignant epithelial liver cell lines. None of the nine fibroblastic or four nonmalignant epithelial cell lines exhibited GGT activity. Our results suggest that by use of GGT activity we can detect in cultured liver cells a significant fraction of the spontaneously or chemically induced malignant cells. Thus, in conjunction with other markers, this marker may help in identifying tumorigenic cells in liver epithelial cultures.

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Mutagenesis and Cell Transformation of Mammalian Cells in Culture by Chemical Carcinogens*

Eliezer Huberman

In the process of *in vitro* cell transformation, normal cells, which have an oriented pattern of growth and a limited life span *in vitro* and which are not tumorigenic, are converted into cells that have a hereditary random pattern of growth, the ability to grow continuously in culture, and the ability to form tumors. Such heritable phenotypic changes may arise from alterations in gene expression due to somatic mutations after interaction of the carcinogen with cellular DNA. Our studies have indeed shown that (*a*) metabolically activated carcinogenic polycyclic hydrocarbons, which have been shown to bind to cellular DNA, induce somatic mutations in mammalian cells; (*b*) there is a relationship between the degree of mutant induction and the degree of carcinogenicity of the different hydrocarbons tested; and (*c*) the somatic mutations were induced by metabolites rather than by the hydrocarbons themselves. In the case of benzo[*a*]pyrene, a very common carcinogenic polycyclic hydrocarbon, its 7,8-diol-9,10-oxide was identified as the major mutagenic and cell-transforming metabolite. Based on these studies, it was possible to estimate the genetic target size for cell transformation by comparing in the same cells the frequency of cell transformation and mutation for ouabain resistance (which is presumably due to a mutation at one locus) induced by benzo[*a*]pyrene and by one of its major metabolites. The results indicated that the target size for transformation is 20 times larger than that determined for ouabain resistance. This suggests that cell transformation, as determined by a hereditary pattern of cell growth, may be due to a mutation and that this mutation can occur in one out of a small number of the same or different genes.

*Research sponsored jointly by the National Cancer Institute and the Division of Biomedical and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with Union Carbide Corporation.

Cell-Mediated Mutagenesis and Cell Transformation by Chemical Carcinogens

Eliezer Huberman and Robert Langenbach

In the process of *in vitro* cell transformation, normal nontumorigenic cells, which have an oriented pattern of cell growth and a limited life span in culture, are converted into tumorigenic cells, which have a hereditary random pattern of cell growth and an infinite life span in culture. Such heritable phenotypic changes may arise from mutational events resulting from an interaction of a carcinogen with cellular DNA.

It is, therefore, important to study mutagenesis induced in mammalian cells by chemical carcinogens. Our studies have shown that mutagenesis of mammalian cells can be achieved by carcinogenic polycyclic hydrocarbons, nitrosamines, and aflatoxins when tested in the presence of fibroblasts and hepatocytes which are able to metabolize these carcinogens. Further, we have found that there is a relationship between the degree of mutant induction and the degree of carcinogenicity of the different chemicals tested. By simultaneously measuring the frequency of cell transformation and the frequency of mutation at one locus (ouabain resistance) in the same cell system, it was possible to estimate the genetic target site for cell transformation. The results indicated that the target site for transformation is approximately 20 times larger than that determined for ouabain resistance. The results suggest that cell transformation may be due to a mutational event and the mutation can occur in one out of a small number of the same or different genes, and that the cell-mediated mutagenesis approach may be a valuable means of detecting tissue-specific carcinogens.

Analysis of Proteins of Mouse Sarcoma Pseudotype Viruses: Type-Specific Radioimmunoassays for Ecotropic Virus p30s*

Stephen J. Kennel and Raymond W. Tennant

Murine sarcoma virus pseudotypes were prepared by infection of transformed nonproducer cells, A1-2, with prototype N-tropic (Gross), B-tropic (WN1802B), or NB-tropic (Moloney) viruses. Proteins from virus progeny were analyzed on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Bands present in both the 65,000- and the 10,000–20,000-mol. wt regions of the gel distinguished the pseudotype viruses from their respective helpers. Furthermore, two protein bands were noted in the p30 region of murine sarcoma virus (Gross), one corresponding to Gross virus p30 and another of slightly slower mobility. Type-specific radioimmunoassays for Gross virus p30 and for WN1802B p30 were developed for analysis of pseudotype preparations. Among several ecotropic viruses tested, only the homologous virus scored in the respective assay. By use of these assays, pseudotype viruses were found to contain only 8–48% helper-specific p30s; the remainder is presumably derived from sarcoma virus. The presence of sarcoma-specific p30 in pseudotype viruses with Fv-1 restricted host ranges indicates that biochemical inferences about p30 as a target molecule for the action of the Fv-1 gene product require biological verification.

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Metabolic Conversion of 12-*O*-Tetradecanoylphorbol-13-acetate (TPA) in Adult and Newborn Mouse Skin and Mouse Liver Microsomes*

David L. Berry, William M. Bracken, Susan M. Fischer, Aurora Viaje, and Thomas J. Slaga

Tritiated 12-*O*-tetradecanoylphorbol-13-acetate (TPA) was applied to adult mouse skin and, at specified time intervals, the mice were killed and the labeled phorbol was extracted and subjected to separation and quantitation by high-pressure liquid chromatography. After 24 hr, TPA comprised over 96% of the recovered label from the skin, and its apparent half-life was 17.8 hr. Pretreatment of adult skin with TPA for 4 weeks before treatment with labeled TPA resulted in an increase in the clearance rate of TPA from the skin. Skin from newborn mice was capable of converting TPA into monoesters and phorbol, but the clearance rate in the adult was about 12 times more rapid than in the newborn. Epidermal homogenates converted TPA into 12-*O*-tetradecanoylphorbol, phorbol-13-acetate, and phorbol. Hepatic homogenates were able to convert TPA to monoesters and phorbol at rates 14–15 times faster than epidermal homogenates. Attempts to isolate any previously undescribed metabolites of TPA by use of liver homogenates were unsuccessful, and mixed function oxidation did not contribute to the metabolism of TPA. Judging from inhibitor studies, esterases were implicated in the conversion of TPA to monoesters and phorbol. The results support the hypothesis that the tumor-promoting activity of TPA is directly related to its concentration in a specific tissue and that conversion of TPA to an active metabolite probably does not occur.

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Lack of Tumor-Promoting Ability of Certain Environmental Chemicals in a Two-Stage Mouse Skin Tumorigenesis Assay*

D. L. Berry, J. DiGiovanni,† M. R. Juchau,† W. M. Bracken, G. L. Gleason, and T. J. Slaga

The food antioxidants butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were tested as tumor promoters on CD1 female mice initiated with 7,12-dimethylbenz[*a*]anthracene (DMBA). At a dose of 1 mg twice weekly they did not promote skin tumors, nor did they produce tumors when tested as a complete carcinogen without DMBA initiation. The polychlorinated biphenyl Aroclor 1254 (PCB) and the polybrominated biphenyl Firemaster-6 (PBB) were also tested for their ability to promote skin tumors; at a 100 μg dose twice weekly they were inactive. The environmental contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) at a dose of 0.1 μg twice weekly did not promote skin tumors in DMBA-initiated mice. TCDD, PCB, and PBB did not promote spontaneous tumors. None of the compounds at the dosages tested significantly increased the intrafollicular epidermis, nor did they appear to be chronically toxic to the test animals. These results indicate that dosage may be an important factor in promotion, since several of the tested compounds are known to be promoters in pulmonary and hepatic systems.

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Studies with Chlorinated Dibenzo-*p*-dioxins, Polybrominated Biphenyls, and Polychlorinated Biphenyls in a Two-Stage System of Mouse Skin Tumorigenesis: Potent Anticarcinogenic Effects

David L. Berry, Thomas J. Slaga, John DiGiovanni,* and Mont R. Juchau*

Abstract not available.

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Carcinogenicity and Mutagenicity of Benz[*a*]anthracene Diols and Diol Epoxides*

Thomas J. Slaga, Eliezer Huberman, James K. Selkirk, Ronald Harvey,†
and William M. Bracken

Benz[*a*]anthracene (BA) and its five possible *trans*-dihydrodiols were evaluated for determination of their skin tumor–initiating activity and their mutagenic activity in Chinese hamster V79 cells. In addition, the skin tumor–initiating abilities of five diol-epoxides of BA were tested. Results showed that (±)-*trans*-3,4-dihydroxy-3,4-dihydrobenz[*a*]anthracene (BA 3,4-dihydrodiol) was more mutagenic than BA and about 20 times more mutagenic than were the other possible dihydrodiols in the V79 cells co-cultivated with irradiated hamster embryo cells. As a skin tumor initiator, BA 3,4-dihydrodiol was approximately five times more active than BA, whereas the other BA dihydrodiols were all less-active tumor initiators. (±)-*trans*-3a,4β-Dihydroxy-1a,2a-epoxy-1,2,3,4-tetrahydrobenz[*a*]anthracene was found to be approximately 20% more active as a tumor initiator than BA 3,4-dihydrodiol, whereas the other diol-epoxides of BA were less active than BA itself. The results suggest that the bay-region diol-epoxide of BA may be the ultimate carcinogenic and mutagenic form of BA.

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Dibenz[*a*,*c*]anthracene: A Potent Inhibitor of Skin Tumor Initiation by 7,12-Dimethylbenz[*a*]anthracene*

Thomas J. Slaga, Aurora Viaje, Steven G. Buty,† and William M. Bracken

The mechanism by which the weak tumor initiator dibenz[*a*,*c*]anthracene (DB[*a*,*c*]A) inhibits the skin tumor–initiating activity of 7,12-dimethylbenz[*a*]anthracene (DMBA) was investigated. DB[*a*,*c*]A was found to be a potent inhibitor of DMBA initiation when administered either 5 min, or 1, 12, or 36 hr before DMBA. Pretreatment of mice with unlabeled DB[*a*,*c*]A at either 1, 12, or 36 hr before killing increased the *in vitro* epidermally mediated covalent binding of [^{3}H]DMBA to DNA more than pretreatment with unlabeled DMBA at comparable times. Only when the tumor experiments were mimicked did a decrease in DMBA covalent binding to DNA *in vitro* occur. The results suggest that some competition at the level of polycyclic hydrocarbon metabolism or at the genome level may exist between metabolites of the weak carcinogen and those of the strong carcinogen.

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Inhibition of Tumor Promotion of Anti-inflammatory Agents: An Approach to the Biochemical Mechanism of Promotion*

T. J. Slaga, A. Viaje, D. Berry, S. Fischer, W. Bracken, S. LeClerc, and D. Miller

Anti-inflammatory steroids such as dexamethasone (DEX) and fluocinolone acetonide (FA) have been found to be extremely potent inhibitors of tumor promotion. Simultaneous doses as low as 0.01 μg of FA and 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) resulted in an almost complete inhibition of tumor promotion. Likewise, FA was found to be a very potent inhibitor of epidermal DNA synthesis. Even at a dose level of 0.01 μg, FA was still effective in inhibiting epidermal DNA synthesis. FA was less effective in inhibiting epidermal DNA synthesis when the skin was pretreated with TPA in order to prestimulate DNA synthesis as compared with unstimulated skin.

The inhibitory effect of FA on tumor promotion appears to be reversible and more specific for the proliferation step than for the conversion step of promotion. Furthermore, the anti-inflammatory ability of a series of steroids was found to correlate not only with tumor promotion but also with their ability to inhibit epidermal DNA synthesis and TPA-induced cellular proliferation (fluocinonide $\geqslant$ FA $>$ fluclorolone $>$ DEX $>$ cortisol). The clinically used nonsteroidal anti-inflammatory agents oxyphenbutazone, Indomethacin, and Seclazone also inhibited tumor promotion but were much less effective than the steroids. These agents had very little effect on epidermal DNA synthesis and TPA-induced inflammation and cellular proliferation.

The anti-inflammatory steroids are effective in counteracting most of the biochemical changes induced by a promoter. On polyacrylamide gels, DEX reduced the levels of several protein fractions, which were greatly enhanced after TPA treatment. The binding of phorbol-ester tumor promoters to an epidermal cytosol receptor protein and chromatin was markedly suppressed by simultaneous treatment with DEX *in vivo*. In this regard, TPA is not metabolized by skin, nor did FA induce the metabolism of TPA. However, FA was ineffective in counteracting the elevated ornithine decarboxylase activity (ODC) induced by TPA. Ongoing studies on the role of ODC in tumor promotion and on the specific localization of TPA and FA and the effect of FA on TPA binding will be presented.

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Marked Differences in the Skin Tumor–Initiating Activities of the Optical Enantiomers of the Diastereomeric Benzo[*a*]pyrene 7,8-Diol-9,10-epoxides*

T. J. Slaga, W. M. Bracken, G. Gleason, W. Levin,† H. Yagi,‡ D. M. Jerina,‡ and A. H. Conney†

The abilities of the optically pure (+)- and (–)-enantiomers of the diastereomeric 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrenes derived from the enantiomeric *trans*-7,8,-dihydrodiols to initiate skin tumors in mice were determined with a two-stage system of tumorigenesis. As a tumor initiator, (+)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene-2 was approximately 60% as active as benzo[*a*]pyrene, whereas (–)-7α,8β-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[*a*]-pyrene-2 was about 2% as active as benzo[*a*]pyrene. The racemic mixture of the above diol-epoxide, in which the 9,10-epoxide is trans to the 7-hydroxyl group, was 25% as active as BP as a tumor initiator. (–)-7β,8α-Dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene-1 and (+)-7α,8β-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene-1, in which the 9,10-epoxide is cis to the 7-hydroxyl group, were found to have little or no tumorigenic activity. The tumor-initiating ability of (+)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene-2 was found to be greater when given daily for 6 days at a dose of 34 nmol/day than when given once at a 200 nmol dose level. Similar fractionated doses of benzo[*a*]pyrene or (–)-7α,8β-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene-2 did not increase their skin tumor–initiating activity. The data suggests that (+)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene-2 is an ultimate carcinogenic form of benzo[*a*]pyrene.

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In Vitro Transformation of Epidermal Cells from Newborn Mice*

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Cultures of epidermal cells obtained from newborn BALB/c mice were used to study *in vitro* transformation of epithelial cells. One-day-old primary cultures plated at 10^6 cells/ml were treated for 2 days with various concentrations of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), 3-methylcholanthrene (MC), and MC-11,12-epoxide. Untreated newborn epidermal cells both divide and keratinize *in vitro* in medium 199 supplemented with 10% fetal calf serum. After a few weeks, the cells enlarge, show signs of senescence, and die after about 3 months *in vitro*. The epidermal cells treated with either MNNG, MC, or MC-11,12-epoxide went through a similar crisis, appearing as though they too were going to die at 3 months. However, small populations of the remaining cells began to proliferate into colonies that soon grew to confluence. These epithelial cells were characterized by rapid growth, loss of visible keratinization, and subculturability, having been passaged 12 times in contrast to untreated cells, which are not subculturable. Electron microscope studies did not reveal any true desmosomes, but junctional complexes were present in all the cell strains examined. The injection of 10^6 cells from the various cell strains into athymic nude or syngeneic mice resulted in rapidly growing solid tumors that were characterized as highly anaplastic "undifferentiated" tumors.

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‡Predoctoral investigator, Grant CA 09104 from the National Cancer Institute.

The Lack of Involvement of 6-Hydroxymethylation in Benzo[*a*]pyrene Skin Tumor Initiation*

T. J. Slaga, W. M. Bracken, A. Viaje, D. L. Berry, S. M. Fischer, and D. R. Miller†

The skin tumor–initiating activities of benzo[*a*]pyrene (BP), 6-hydroxymethyl benzo[*a*]pyrene (6-OH-CH_2-BP), 6-methylbenzo[*a*]pyrene (6-CH_3-BP) and the effects of 7,8-benzoflavone (7,8-BF), quercetin, and 1-benzylimidazole on their activity were determined in mice using a two-stage system of tumorigenesis. The skin tumor–initiating activity of 6-OH-CH_2-BP and 6-CH_3-BP was determined to be $\frac{1}{8}$ and $\frac{1}{5}$, respectively, of the activity of BP. 7,8-BF had very little effect on the skin tumor–initiating activity of 6-OH-CH_2-BP and 6-CH_3-BP. However, a dose-dependent inhibition of BP tumorigenesis by 7,8-BF was noted. Quercetin and 1-benzylimidazole were also found to inhibit BP skin tumor–initiating activity. Direct hydroxymethylation of BP does not appear to be an important pathway in the activation of BP in mouse skin tumor initiation.

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†Predoctoral investigator, Grant CA 09104 from the National Cancer Institute.

Inhibition of Phorbol Ester-Induced Tumor Promotion by Vitamin A Analog and Anti-inflammatory Steroid

C. E. Weeks, T. J. Slaga, H. Hennings,* G. L. Gleason, and W. M. Bracken

The effects of a vitamin A analog, TMMP ethyl retinoate (Ro 10-9359), and an anti-inflammatory steroid, fluocinolone acetonide (FA), given separately or together were studied in a two-stage carcinogenesis system. The phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA) was employed as the tumor promoter in a 7,12-dimethylbenz[*a*]anthracene (DMBA)-initiated mouse skin system. Two strains of mice, which differ in their degree of sensitivity to skin carcinogenesis, were used. A dose-dependent inhibition of carcinogenic expression, as determined by decreased number of papillomas per animal, was observed in each mouse strain with both FA and Ro 10-9359. When FA and Ro 10-9359 were given together, an enhanced effect on lowering tumor incidence was noted. FA was effective in inhibiting tumor formation in the sensitive mouse strain even when the steroid was given 1 day prior to TPA treatment under conditions of "saturating" doses of initiator (DMBA) and/or promoter (TPA). These results suggest that anti-inflammatory steroids and retinoids not only provide inhibition of tumor promotion but can be effectively utilized in a combination regimen for increased chemopreventive response.

**In Vitro* Pathogenesis Section, Experimental Pathology Branch, National Cancer Institute, Bethesda, Maryland 20014.

Relative Carcinogenic Effectiveness of Derivatives of Nitrosodiethylamine in Rats*

W. Lijinsky† and H. Wayne Taylor‡

The carcinogenicity of five derivatives of nitrosodiethylamine was compared with that of the parent compound by per os administration to rats. All were less potent than was nitrosodiethylamine. When nitrosobis(2-methoxyethyl)amine and nitrosobis(2-ethoxyethyl)amine were administered at equimolar doses in drinking water, there was a high incidence of liver tumors, but the animals died later than they did after nitrosodiethylamine treatment, which also induced esophageal tumors. Nitrosoiminodipropionitrile and nitrosobis(2,2-diethoxyethyl)amine failed to induce tumors at the same dose level. Nitrosobis(2-chloroethyl)amine was administered in oil by gavage at a dose lower than that of nitrosodiethylamine and

produced a much weaker tumor response; 5 of 15 treated rats had forestomach papillomas, and 1 had olfactory adenocarcinoma and no other induced tumors.

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Transfection of FV-1 Permissive and Restrictive Mouse Cells with Integrated DNA of Murine Leukemia Viruses*

Ih-Chang Hsu,† Wen K. Yang, Raymond W. Tennant, and Arthur Brown‡

Whole-cell DNA preparations isolated from SC-1 cells chronically infected with N- or B-tropic murine leukemia viruses (MuLV) were tested for infectious activity in an *Fv-1^n* (NIH-3T3) and two *Fv-1^b* (C57BL/6 and SV-A31) cell cultures. Efficiency of transfection of all DNAs was better in the NIH-3T3 cells than in C57BL/6 or SV-A31 cells; and the [N-tropic MuLV] SC-1–cell DNA preparation was slightly more infectious than the [B-tropic MuLV] SC-1–cell DNA preparation in all three cell cultures, regardless of their Fv-1 genotypes. Progeny viruses from the tranfection showed N- or B-tropism corresponding to that of the parent viruses produced by the infected SC-1 cells that were used for the DNA preparation. DNA dose-response studies in NIH-3T3 cells revealed a one-hit mechanism for both the [B-tropic MuLV] SC-1–cell DNA and the [N-tropic MuLV] SC-1–cell DNA preparation. These results demonstrate that, in contrast to virion infection, transfection of N- or B-tropic MuLV with DNA preparations from chronically infected cells is not affected by the Fv-1 gene.

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In Vitro Binding of Selective Transfer RNAs to 18S and 28S Ribosomal RNAs of Mouse Cells*

David L. R. Hwang,† Wen K. Yang, James O. Kiggans, Jr., and Claude D. Stringer

In an *in vitro* hybridization reaction, 18S and 28S ribosomal RNAs of BALB/c mouse cells are able to bind certain species of tRNA. The rRNA·tRNA complexes can be isolated and the hybridized tRNAs, after heat dissociation, can be identified by amino acid–accepting activities. Using this method, we found that 18S rRNA selectively bound $tRNA^{Lys}$, $tRNA^{Gly}$, $tRNA^{Glu}$, and $tRNA^{Ala}$, whereas 28S rRNA bound predominantly $tRNA^{Pro}$ and subordinately $tRNA^{Gly}$, $tRNA^{Ala}$, and $tRNA^{Asp}$. All tRNAs, except $tRNA^{Lys}$, in the hybridized state, were unable to accept amino acids in the synthetase-catalyzed reaction; heat dissociation restored the amino acid–accepting properties. The dissociation temperature of the 28S rRNA·tRNA complex was 59°C, and that of the 18S rRNA·tRNA complex was 62°C in a 0.1 *M* NaCl solution. With excess tRNAs included in the hybridization mixture, the tRNA-binding capabilities of rRNAs prepared from young adult mouse livers were determined to be 0.05 mol $tRNA^{Glu}$ per mole of 18S rRNA and 0.1 mol $tRNA^{Pro}$ per mole of 28S rRNA. Binding of different tRNAs on the rRNA molecules appeared to be at different sites, as indicated by the binding capability of fragmented rRNA molecules.

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†Postdoctoral investigator, subcontract 3322 from the Biology Division of Oak Ridge National Laboratory to the University of Tennessee.

Binding of Immunoglobulin G to Phospholipid Vesicles by Sonication*

Leaf Huang† and Stephen J. Kennel

Purified goat immunoglobulin G to murine leukemia virus protein, gp-70, does not bind to the sonicated phospholipid vesicles. However, when immunoglobulin is sonicated together with phospholipids, 4–95% of the immunoglobulin can be bound to the vesicles, depending on the experimental condition. The extent of binding depends on the period and power of sonication, the IgG to lipid ratio, and the lipid composition. Anionic phospholipids such as phosphatidylglycerol and phosphatidylserine, but not cholesterol, enhance binding about 50% over the neutral phosphatidylcholine. Binding of immunoglobulin causes extensive aggregation of vesicles, as shown by electron microscopy, so that the aggregates can be separated from unbound immunoglobulin by molecular-sieve chromatography on Sepharose 4B. The immunoglobulin-vesicle aggregates remain stable in either phosphate-buffered saline or 50% fetal calf serum up to 20 hr at 37°C, although substantial lipid degradation in 50% fetal calf serum was observed. Vesicle-bound immunoglobulin retains 30–50% of the original antigen binding capacity by a radioimmunoassay, whereas the immunoglobulin sonicated alone shows 100%. Identical antigen binding capacity of bound immunoglobulin is found when vesicles are lysed by 1.5% NP-40, suggesting all of the bound immunoglobulins are exposed to the outer surfaces of the vesicles. Purified $(Fab)_2$ fragment of the immunoglobulin also binds with vesicles by sonication.

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Endogenous Primate Type C Viral Gene Expression: Differential Induction by 5′-Bromodeoxyuridine*

George Lavelle, Stephen J. Kennel, and Richard L. Heberling†

Fetal diploid baboon fibroblasts were induced with 5′-bromodeoxyuridine to synthesize baboon type C viral GAG gene product, p28. Intracellular BaEV p28 antigen was detected by specific immunofluorescence. Induction frequencies as high as 5×10^{-2} were observed. Different strains of baboon cells produced little or no extracellular viral RNA-directed DNA polymerase or infectious virus after induction. Thus, GAG gene was differentially expressed. High-frequency differential induction of endogenous retroviral genes in primate cells has not been previously recognized.

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Postintegration Restriction of Baboon Endogenous Virus in Exogenously Infected Baboon Cells*

G. Lavelle, L. Foote, R. L. Heberling,† and S. S. Kalter†

Strains of low-passage, fetal baboon cells were examined for their expression of endogenous baboon type C virus (BaEV) and for their susceptibility to exogenous infection. Whole baboon embryo (WBE) cells spontaneously and chronically released low levels of infectious virus. Fetal skin-muscle (FSM) cells were negative for virus expression by immunofluorescence tests for viral p28, by supernatant RNA-directed DNA polymerase (RDDP) assays, and by infectivity of culture fluids and co-cultivation with heterologous cells. FSM cells, however, along with two other strains of baboon cells, were susceptible to exogenous infection with three independent isolates of BaEV. Infectivity of the M7 strain of BaEV for FSM cells was equivalent

to that for human and dog cells in that similar linear, single-hit titration patterns were obtained as measured by viral p28 immunofluorescence. By RDDP assays, however, baboon cells produced only low levels of virus after infection compared with production by heterologous cells. Synthesis of viral p28 accompanied by small amounts of infectious virus suggests that restriction probably occurs after provirus integration at the level of transcription or translation.

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Defective C-Type Retrovirus Particles Secreted by L1210 Leukemia Cells

S. J. Kennel, F. Tsakeres, P. A. Kelly, and D. P. Allison

Cultures of 1210 leukemia cells (L1210) have been found to release C-type retrovirus. Because L1210 is used as a common model system in cancer research, the potential influence of these virus particles in the course of tumor cell growth was studied. The virus particle was found to be nononcogenic and noninfectious in standard assays for xenotropic and ecotropic murine retroviruses. The virus, designated as L1210 virus, had a normal complement of viral proteins except that it had no detectable gp70. Quantitative radioimmunoassay for the major structural protein, P30, indicated that the virus is at least 1000-fold more defective than ecotropic viruses such as AKR and Moloney leukemia virus.

L1210 cells grown *in vitro* secreted about 20-fold more particle-associated P30 than did *in vivo* cultured cells, but cell-associated P30 and gp70 were found at similar levels. Both *in vitro* and *in vivo* cultured cells displayed gp70 on their surfaces which appeared to be of larger molecular weight than gp70s from most ecotropic viruses. Cells grown *in vitro* released a high level of non-virion-associated gp70 into the growth medium, but the high concentration of gp70 in the serum of normal DBA/2 mice precluded such measurements on *in vivo* cultured cells. These results indicate that L1210 virus does not act as a classical infectious leukemia virus during growth of L1210 cells, but the presence of relatively large amounts of viral antigens in these cells may affect the immune response to them.

Identification and Characterization of a Tumor-Specific Protein from a BALB/c Lung Carcinoma

Stephen J. Kennel

Line 1, a spontaneous alveolar carcinoma from a BALB/c mouse, is highly metastatic and weakly antigenic in the syngeneic host. Xenogeneic antisera were prepared for study of the interaction between this tumor line and the host's immune system and for use as reagents for immunotherapy experiments. Sera and immunosorbent-enriched antibody preparations were made specific for line 1 cells by *in vitro* and *in vivo* absorptions. Lactoperoxidase-catalyzed radioiodination of cell-surface protein followed by precipitation with specific antibodies identified a protein of about 180,000 mol. wt (designated TSP-180) present on line 1 cells but not on normal lung cells or Moloney sarcoma tumor cells. This tumor-specific protein did not bind to Concanavalin A and was not precipitated by antisera to leukemia virus proteins, filamin, carcinoembryonic antigen, or large external transformation sensitive protein.

Activation of Murine Leukemia Virus in Cell Culture by Irradiation

James A. Otten

Abstract not available.

Involvement of DNA Damage in Hydroxyurea-Mediated Induction of Endogenous Murine Retrovirus*

Ralph J. Rascati and Raymond W. Tennant

Hydroxyurea (HU) induces AKR cells to produce endogenous murine retrovirus at a low frequency (1×10^{-5}), and DNA synthesis is required soon after treatment with HU for induction to be observed (i.e., no stable induction intermediate is formed). Induction by HU can be enhanced by simultaneous treatment with halogenated pyrimidines, with the concomitant appearance of a stable provirus intermediate. The effects of the two compounds are synergistic, indicating an actual stimulation of HU-mediated induction by iododeoxyuridine. Since HU inhibits semiconservative replication and since [^{3}H]bromodeoxyuridine is incorporated into the cellular genome predominantly by unscheduled DNA synthesis (repair replication) under these conditions, this stimulation appears to be the result of insertion into DNA of the thymidine analogs during the repair of HU-induced alterations in the DNA. The nature of HU damage to DNA is not defined; if single-strand breaks are involved they may occur at a frequency $<10^{-8}$ and escape detection, but induction could also be due to other alterations in DNA. The characteristics of induction by HU, therefore, are similar to those of induction by other DNA-damaging treatments such as γ- or X-irradiation or methylcholanthrene. This suggests that these agents may induce by similar, if not identical, mechanisms. Further, the ability of halogenated pyrimidines to form a stable induction intermediate when incorporated by repair synthesis, similar to the intermediate formed when the analogs are incorporated during semiconservative replication, suggests that the same sites are involved for induction by damaging agents or by halogenated pyrimidine incorporation.

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Selective Growth of Some Rodent Epithelial Cells in a Medium Containing Citrulline

N. C. Sun, C. R. Y. Sun, A. W. Hsie, and R. W. Tennant

We have defined a medium (called Sun's modified Waymouth medium) which selectively cultures some rodent epithelial cells that are capable of utilizing citrulline in place of arginine. A growth-response study of the ability of 47 different mammalian cell cultures (of mouse, rat, Syrian hamster, Chinese hamster, guinea pig, rabbit, monkey, and human origin) to utilize arginine or its biosynthetic precursors ornithine, citrulline, or argininosuccinate showed that all epithelial cells and some fibroblasts are capable of growing in citrulline medium; however, primary embryo fibroblasts and 12 established fibroblast cell lines derived from Syrian hamster failed to grow. The citrulline medium also allowed selective outgrowth of epithelial cells, without contaminating fibroblasts, from Syrian hamster tracheal explants. This absolute nutritional difference between Syrian hamster epithelial and fibroblast cells allows citrulline medium to be used for selective cultivation of epithelial cells.

Chimpanzee Chromosome 13 Is Homologous to Human Chromosome 2p

N. C. Sun, C. R. Y. Sun, and T. Ho

The chimpanzee (*Pan troglodytes*) has 48 chromosomes as opposed to a human's 46 chromosomes, but the banding patterns of most human chromosomes are extremely similar to the chimpanzee's, except the human chromosome 2 could not find its counterpart in chimpanzee.

In (1*s* + 1*s*) interspecific hybrid between human lymphoblastoid cell and galactose-negative mutant, Gal-2, of Chinese hamster origin, a single human chromosome 2 is preferentially retained because the *Gal*$^+$-*Act* gene or the gene for NADH coenzyme reductase is located on human chromosome 2p, which complements the defectiveness of Gal-2 mutant. By fusing Gal-2 cells with chimpanzee lymphoblastoid cells, three

primary hybrid clones have been isolated and shown to exhibit both chimpanzee and Chinese hamster forms of MDH_S and ACP_1. The chimpanzee chromosome 13 (Paris Conference, Supplement) was always retained in the hybrids maintained in selective galactose medium, which eliminates the parental Gal-2 cells.

One of the hybrids, GC-1, was employed to study the relationship of the presence of chimpanzee chromosome 13 and the concordant expression of MDH_S, ACP_1, and galactose utilization. In 20 positive subclones able to grow in galactose medium, chimpanzee forms of MDH_S and ACP_1 were expressed, while ten negative subclones unable to grow in galactose medium did not express chimpanzee forms of MDH_S and ACP_1. As a result, *MDH_S*, *ACP_1*, and *Gal^+-Act* genes can be assigned to chimpanzee chromosome 13. Since *MDH_S*, *ACP_1*, and *Gal^+-Act* genes have been assigned to human chromosome 2p, we suggest that chimpanzee chromosome 13 is homologous to human chromosome 2p. Our conclusion is different from what was tentatively proposed by Paris Conference, Supplement, and also different from what was hypothesized by de Grouchy *et al.*

Chimpanzee Chromosome 12 Is Homologous to Human Chromosome 2q

N. C. Sun, C. R. Y. Sun, and T. Ho

According to chromosome banding patterns, most of the human 46 chromosomes find their counterparts in the chimpanzee's 48 chromosomes except for chromosome 2, which has been hypothesized to have been derived from a centric fusion of two chimpanzee acrocentric chromosomes. These two chimpanzee chromosomes have been suggested as 13 and 12.

Since the *GALT* gene was first localized on human chromosome 2 and further regionally localized to the centromeric region of this chromosome (2q11→2q14), we considered it helpful to use *GALT* as an isozyme marker to trace the homologue of human chromosome 2q in the chimpanzee genome.

Cell lysates of 48 subclones from GC-14, a hybrid clone of Chinese hamster Gal-2, and chimpanzee LE-7 cells were prepared and examined by starch gel electrophoresis for the presence of the chimpanzee form of *GALT* activity. The isozyme patterns observed suggested that the chimpanzee *GALT* was expressed in ten of the 48 subclones. Detailed karyotype analysis and *GALT* expression of the primary hybrids of GC-1, GC-4, GC-10, and GC-14, and some of the subclones of GC-14 are shown. The correlation of the expression of the chimpanzee form of *GALT* in all these clones with the presence of chimpanzee chromosome 12 suggests that the *GALT* gene is located on chimpanzee chromosome 12 and that this chromosome is homologous to human chromosome 2q.

Specific Cell RNA Mediators and the Mechanism of *Fv-1* Gene Restriction*

Raymond W. Tennant, Wen K. Yang, Ralph J. Rascati, I. C. Hsu, and Arthur Brown†

Cells that are normally permissive for both N- and B-tropic viruses (*$Fv\text{-}1^{--}$*) can be made resistant to these viruses by treatment with phenol-extracted fractions of mouse cells with the appropriate *Fv-1* genotype (*$Fv\text{-}1^{bb}$* or *$Fv\text{-}1^{nn}$*). The ability of RNase, but not DNase or pronase, to inhibit resistance transfer indicated that resistance is mediated by an RNA molecule. The assay for resistance transfer is based on XC-plaque reduction in DEAE-dextran treated cells. By this assay method, stored pools of cellular RNA have shown statistically significant specific activity at concentrations of 5–10 μg. However, the maximum plaque reduction is 60–80% and appears to be related to the ability of cells to incorporate RNA. Specific activity has been localized in the 18–22S region of sucrose gradients. In addition, it can be detected in H_2O eluates from poly(U)Sepharose columns, suggesting that the RNA is polyadenylated. The time course of resistance transfer indicates that the restriction mediated by RNA functions only if added within 6 hr after virus infection.

By the technique of DNA transfection, high-molecular-weight DNA extracted from SC-1 cells infected with either N- or B-tropic virus have been found to infect cells of *Fv-1*^{nn} and *Fv-1*^{bb} genotypes equally well. Similar findings have been obtained with the Hirt extract supernatant DNA isolated from the infected SC-1 cells. It has not been possible to obtain infectious DNA of nonpermissive cells infected at similar multiplicities of infection. These results, therefore, indicate that the *Fv-1* locus causes synthesis of biologically defective provirus.

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Mechanisms of Cocarcinogenesis Involving Endogenous Retroviruses*

Raymond W. Tennant and Ralph J. Rascati

Interactions between chemical carcinogens and tumor viruses generally lead to a higher incidence of tumors in animals exposed to both agents than in animals exposed to either agent alone. However, the role of endogenous retroviruses in carcinogenesis is as yet unclear and is complicated by the existence of many host-range types of viruses with varied organ specificities. Recent work with radiation leukemia virus demonstrates the complexities of this system.

Endogenous viruses can be induced by a variety of agents including thymidine analogs, protein synthesis inhibitors, and amino acid analogs. The mechanism of induction is not known, but evidence suggests that a repressor-like substance is involved and that inducing agents interfere with either the synthesis of this substance or its action. In addition, endogenous retrovirus expression has also been induced by physical carcinogens such as γ- and X-radiation, and also by other agents that damage DNA. Virus is also induced by hydroxyurea by an unknown mechanism which may involve subtle damage to the DNA. Other carcinogens such as 3-methylcholanthrene and 7,12-dimethylbenz[*a*]anthracene can also induce endogenous retrovirus expression. The evidence suggests that physical and chemical carcinogens induce virus expression by causing damage to DNA and that repair of such damage prior to replication through the area of the lesion abrogates induction. The inductive effect can be potentiated either by inhibition of repair to prolong the existence of the lesion or by incorporation of thymidine analogs during repair synthesis to create a stable induction intermediate, which results in virus expression during a subsequent cycle of semiconservative replication.

Many carcinogens appear to transform cells in culture in the absence of virus induction, but it is entirely possible that certain viral genes were induced in those situations in the absence of production of whole infectious viruses. It must be realized, however, that this is based on our limited ability to identify endogenous viruses and virogene products. The actual situation may involve subtle regulation of only those virogenes responsible for oncogenesis in the absence of identifiable viral products. Since the putative products of the viral transformation (*onc*) gene have not yet been conclusively identified, their participation in chemical and physical carcinogenesis remains uncertain.

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In Vitro Association of Selective Transfer RNA Species with 28S RNA of Mouse Cells*

Wen K. Yang, David L. R. Hwang,† J. O. Kiggans, Jr., D. M. Yang, C. D. Stringer,
D. J. Moore, and F. C. Hartman

28S RNA prepared either from the poly(A) RNA-depleted fraction of mouse embryo culture cells or from 60S ribosome subunits of adult mouse liver is able to bind selective species of tRNAs in an *in vitro* hybridization reaction. The bound tRNA consists predominantly of proline tRNA and, in minor amounts, glycine, alanine, and aspartic acid tRNAs. Quantitative analysis revealed that the hybridization of tRNA may involve a 28S RNA subpopulation, which is present in higher quantity in embryo cells than in adult liver of the mouse.

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Transfer of *Fv-1* Locus-Specific Resistance to Murine N-Tropic and B-Tropic Retroviruses by Cytoplasmic RNA*

Wen K. Yang, Raymond W. Tennant, Ralph J. Rascati, James A. Otten, Bonnie Schluter,†
James O. Kiggans, Jr., Fred E. Myer, and Arthur Brown†

A standardized bioassay for transfer of *Fv-1* gene-specific resistance to N-tropic and B-tropic murine retroviruses was developed using XC-plaque reduction in SC-1 ($Fv\text{-}1^{1-}$) cells inoculated with virus. Testing of subcellular fractions of restrictive cells showed that the resistance transfer activity was present in the cytoplasmic (microsomal and cytosol) fractions. The activity of the cytoplasmic extract was destroyed by treatment with ribonuclease, but not with deoxyribonuclease or proteases. RNA prepared by phenol:chloroform extraction of mouse tissues, including embryos and livers of weanling mice, transferred *Fv-1* locus-specific resistance into DEAE-dextran-treated SC-1 cells. The activity of isolated RNA preparations against virus of the appropriate host-range type has been demonstrated to correspond to the *Fv-1* genotypes of the cell sources. The specific transfer of resistance with cellular RNA was effective within a 5–6 hr period from 2 hr before to 4–5 hr after virus infection. Sucrose gradient centrifugation of the RNA showed that the activity sedimented as a broad peak, with an apparent maximum in the 22S region. Affinity chromatography of whole-cell RNA on poly(U)-Sepharose separated most of the activity into the poly(A) RNA fraction. Except for the reciprocal inhibitory activity for the two host-range virus types, the RNAs of $Fv\text{-}1^{n}$ and $Fv\text{-}1^{b}$ specificities showed similar properties in all aspects studied.

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The Rat Tracheal Transplant: An *In Vivo* Model for Studying the Chemistry and Secretion of Mucus Glycoproteins*

J. N. Clark† and A. C. Marchok

Rat tracheal grafts were placed subcutaneously on the backs of isogeneic rats and established for 4 to 6 weeks. Histological evidence demonstrated that a normal mucociliary epithelium and mucus secretion were maintained for the duration of the experiment. Several milligrams of secretions were obtained from each transplant. These were solubilized and partially purified by column chromatography. Sepharose CL-6B separated the high-molecular-weight mucins (unpurified mucin fraction), which were excluded from the gel,

from most of the serum-type glycoproteins and proteins, including albumin. A reductive-alkylation treatment of the unpurified mucin fraction removed contaminating peptide and mannose-containing material from the mucin fractions. Chemical analysis of the purified mucin fraction showed it to contain 16.5% protein and primarily galactose, *N*-acetylglucosamine, and *N*-acetylgalactosamine in the carbohydrate moiety. The presence of sialic acid and sulfate suggests that this fraction contains sialo- and sulfomucins.

The usefulness and advantages of the tracheal transplant model system for the study of pure tracheal mucins under normal and pathological conditions are discussed.

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Characterization of Mucin Isolated from Rat Tracheal Transplants*

Jeffrey N. Clark,† Ann C. Marchok, and Paul Nettesheim

Tracheal mucins in milligram quantities have been isolated from secretions of rat tracheal transplants using mild solubilizing techniques. Sepharose CL-6B chromatography separated the mucins, which eluted at the void volume, from smaller glycoproteins and contaminating serum proteins, which eluted as three included volume peaks. All peaks were chemically characterized for apparent molecular weight and for sugar, amino acid, and sulfate content, and were subjected to polyacrylamide gel electrophoresis. The mucin fraction consisted of at least 50% carbohydrate, with only small amounts of fucose and xylose and no uronic acid. Hexosamine, sialic acid, galactose, and mannose were present in the following molar ratios: 8.91:1.46:2.60:1.00. Sulfate content was about 6% by weight. This mucin fraction appeared to undergo a β-elimination reaction under mild alkali conditions. Further attempts to purify these tracheal mucins on Sepharose CL-2B or on agarose gel electrophoresis were unsuccessful, but a method to isolate and partially purify subunits derived from the mucin fraction by reductive alkylation is described.

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†Postdoctoral investigator, Carcinogenesis Training Grant CA 05296 from the National Cancer Institute.

Biosynthesis of Glycoproteins by Cultures of Rat Tracheal Explants*

Jeffrey N. Clark,† Ann C. Marchok, and Paul Nettesheim

Secretions labeled by various glycoprotein precursors were obtained from 9-day-old rat tracheal explants cultured in a system that maintains normal morphology for several months. These secretions were fractionated separately on Sepharose CL-6B and DEAE-Sephadex A-50 columns into several peaks which eluted similarly to material derived from a short-term incubation of freshly isolated tracheas. Also, the explant secretions chromatographed similarly on Sepharose CL-6B to secretions obtained from *in vivo* rat tracheal transplants, which have been shown to consist of mucins and other glycoproteins.

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†Postdoctoral investigator, Carcinogenesis Training Grant CA 05296 from the National Cancer Institute.

Evaluation of Morphological Features as Markers for Oncogenic Transformation in Respiratory Tract Epithelial Cells

C. A. Heckman and A. C. Olson

The purpose of the present studies was to determine whether changes in cell shape and microvillar density accompanied oncogenic transformation of rat respiratory tract epithelial cells. Two cell lines that became oncogenic during *in vitro* culture (1000 W and 165 S) were studied. In relatively late passages, but not in early passages, the lines produced keratinizing squamous cell carcinomas when tested in syngeneic hosts. Small colonies, predominately of clonal origin, were obtained at early and late times after initiation of the lines into *in vitro* culture. Scanning electron microscopic studies showed that preoncogenic and oncogenic populations differed with respect to the shapes of cells within colonies. Differences in cell shape were further analyzed by estimation of the height and the ratio of length to width for 20 cells sampled from each colony. Each cell was assigned to one of nine classes of cell shape. The frequency with which spindle-shaped cells were observed in colonies increased threefold with oncogenic transformation of the 1000 W and 165 S lines. The frequency did not increase during *in vitro* culture of a third highly oncogenic cell line, BP 3-0. The frequency of observation of spindle-shaped cells in the 1000 W line was not decreased by *in vivo* growth and rederivation. In fact, the tumor-derived subline, 1000 WT, had a fivefold greater frequency of expression than an early passage of the 1000 W line. The number of colonies in which this cell shape was observed also increased fivefold and came to include nearly half of the colonies analyzed. Therefore, expression of spindle shape became prevalent in clonal subpopulations of the line. In early passages of the 1000 W and 165 S lines, most spindle-shaped cells were found at the edges of colonies. This observation suggested that the spindle shape was assumed in response to forces generated during colony expansion. The number of cells in a second shape class, that of flattened spindle shape, increased specifically at the edges of colonies from the 1000 W line. Representation of this shape class at the edges of colonies increased three- to fourfold in the late 1000 W line and in the 1000 WT subline, compared with an early passage of the 1000 W line. Representation in this class increased only slightly in the 165 S line. In general, the 1000 W line, which was more oncogenic than the 165 S line, also showed more pronounced morphological alterations. The prevalence of ruffles was well correlated with oncogenicity in the 1000 W line. However, the cell lines differed with respect to the density of microvilli at the cell surface, and this feature did not seem well correlated with oncogenicity. The results suggested that cytoskeletal and/or adhesive mechanisms implicated in shape maintenance were altered in parallel with oncogenic transformation of epithelial cells originating from the respiratory tract.

Morphological Characteristics of Soft Agar-Derived Colonies and Sublines from Rat BP 1-0 Cells

C. A. Heckman and S. M. LeClerc*

Cells of an anchorage-independent rat sarcoma cell line were compared with primary, normal rat fibroblasts which required substrate attachment for growth. When cultured on plastic substrata, BP 1-0 cells appeared more rounded and more deformable than normal cells. Long, slender microvilli or blebs were observed on the surface of some BP 1-0 cells but not on the surface of normal fibroblasts. The variability in surface features and cell shape of BP 1-0 cells was not reduced in agar-grown colonies or in sublines obtained from such colonies. Cultures of sublines contained numerous spherical cells, not all of which were in the mitotic phase of the cell cycle. The results suggest that anchorage independence may be correlated with rounded cell shape and surface deformability.

*Predoctoral investigator, ERDA Training Grant E(40-1)4969 to the University of Tennessee.

In Vitro Development of Oncogenicity in Cell Lines Established from Tracheal Epithelium Preexposed *In Vivo* to 7,12-Dimethylbenz[*a*]anthracene*

Ann C. Marchok, Joyce C. Rhoton, and Paul Nettesheim

The purpose of these studies was to determine whether carcinogenesis initiated *in vivo* in tracheal epithelium of rats progresses *in vitro* upon culturing of the epithelium. Transplanted rat tracheas were exposed to either 150 or 640 μg of 7,12-dimethylbenz[*a*]anthracene (DMBA) over a period of 2 weeks. Epithelial cell lines were derived from tracheas exposed in this manner and were tested periodically *in vivo* for development of oncogenicity and for phenotypic changes associated with neoplastic transformation *in vitro*.

Three cell lines were obtained from tracheas preexposed to 150 μg of DMBA. Tumorigenicity was demonstrated only in the late passages of two cell lines maintained for 400 days or more *in vitro*. With these cells, *in vivo* tumor latency was 115 to 255 days. Eight of the nine cell lines derived from tracheas preexposed to 640 μg of DMBA have become tumorigenic. Several of these were found to be oncogenic after approximately 200 days *in vitro* and yielded palpable tumors within 9 to 80 days after inoculation. In several instances, inoculation of cells from early passage into immunosuppressed isogenic recipients resulted in the development of keratinic cysts or tumors that regressed. Inoculation of later passages of the same cell lines resulted in the development of invasive tumors.

Loss of anchorage dependence of growth was, in most cases, a reliable phenotypic marker for development of oncogenicity, as was colony-forming efficiency of plastic. Growth rate, saturation density, and cell and colony morphology were not. All of the cell lines were epithelial in nature, as indicated by ultrastructural characteristics, the production of keratin *in vitro* and/or the formation of squamous cell carcinomas upon inoculation *in vivo*. The cell lines had stable morphological characteristics distinguishing each cell line from any other by growth pattern, cell shape, and degree of keratinization.

Our data indicate that the process of carcinogenesis initiated in epithelial cells *in vivo* continues in *in vitro* culture. Thus it should be possible to study in detail both the evolution of the neoplastic process and factors affecting its progression.

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Vitamin A and the Susceptibility of Respiratory Tract Tissues to Carcinogenic Insults*

P. Nettesheim, C. Snyder, and J. C. S. Kim†

The influence of vitamin A on the development of chemically induced lung carcinomas in rats was investigated. Rats were maintained on low, "normal," and excess levels of retinyl acetate (RA). Respiratory tract squamous carcinomas were induced by intratracheal injections of 3-methylcholanthrene (3-MCA). The carcinogen doses used ranged from 1.25 to 10.0 mg of 3-MCA. Serial sacrifices conducted during the first 20 weeks following carcinogen exposure showed that metaplastic lung nodules – presumed to be precursors of later appearing carcinomas – occurred earlier and at a higher incidence in rats maintained on low levels of RA than in rats maintained on moderate or high levels of RA. The development of invasive pulmonary carcinomas was enhanced at all four carcinogen doses in rats receiving low levels of RA as compared to rats receiving moderate or high levels of RA. No consistent difference in lung cancer incidence existed between the groups receiving normal and high levels of RA. The data clearly show an increased susceptibility of vitamin A–deficient rats to develop chemically induced lung cancers. Possible mechanisms underlying this effect are discussed.

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Studies with a New Experimental Model in Respiratory Carcinogenesis*

Paul Nettesheim and Tsutomu Yarita†

Abstract not available.

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Exposure of Cultured Tracheal Epithelium to 12-*O*-Tetradecanoyl-phorbol-13-acetate Results in the Establishment of Epithelial Cell Lines*

Vernon E. Steele,† Ann C. Marchok, and Paul Nettesheim

In vitro exposure of rat tracheal epithelium to the tumor-promoting agent 12-*O*-tetradecanoyl-phorbol-13-acetate results in a marked increase in growth capacity. The growth changes are manifested in an increased rate of cell division and growth in primary cultures and in the establishment of permanent epithelial cell lines. Such changes did not occur in control cultures. Fourteen of the cell lines have been inoculated into immunosuppressed recipients and all are nontumorigenic.

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†Postdoctoral investigator, Carcinogenesis Training Grant CA 05296 from the National Cancer Institute.

Repopulation of Denuded Tracheal Grafts with Normal, Preneoplastic, and Neoplastic Epithelial Cell Populations*

Margaret Terzaghi, Paul Nettesheim, and Mary Lou Williams

The purpose of these experiments was to study the *in vivo* growth characteristics of nonmalignant and malignant epithelial cell cultures maintained *in vitro* for various lengths of time. All cell cultures tested originated from normal or carcinogen-exposed tracheal epithelium of Fischer-344 rats. They were inoculated into isolated tracheas which were then grafted to isogenic recipients. At least 10^4 epithelial cells were required to reestablish a complete epithelial lining in denuded tracheal grafts, whether normal or tumorigenic cell cultures were used. Inoculation of denuded tracheas with 1-week-old primary cultures resulted in the reestablishment of a near-normal mucociliary tracheal lining within 1 to 2 weeks. Injection of primary epithelial cultures older than 1 week failed to reestablish an epithelial lining; instead, the tracheal lumen became obliterated with connective tissue. Inoculation with preneoplastic epithelial cell lines resulted in the establishment of a well-organized, keratinizing squamous epithelium, which remained stable for at least 6 weeks if the recipient was immunosuppressed, but which was rejected at 4 weeks in immunocompetent hosts. Inoculation with a neoplastic cell line resulted in the establishment of a well-organized squamous epithelium for 3 to 4 weeks and development of disorganization, exophytic growth, and invasion at 4 to 6 weeks. This occurred only in immunosuppressed recipients. In immunocompetent hosts the epithelial lining was completely rejected within 2 weeks. When cells from a highly malignant squamous cell carcinoma line were inoculated into tracheas with an intact epithelial lining, only a few isolated nests of malignant cells were observed at 6 weeks. Repopulation of denuded tracheas with cells from the same tumor line resulted in establishment of an atypical squamous lining at 1 week. At 2 weeks invasion was widespread, resulting in destruction of the tracheal grafts shortly thereafter.

The epithelial morphologies observed in these studies are highly reminiscent of various preneoplastic and neoplastic "lesions" induced in tracheas of rats by direct action of chemical carcinogens. Our experiments show that this "*in vivo* culture system" is well suited to the study of growth and differentiation characteristics of carcinogen-altered or preneoplastic epithelial cell populations. This is not easily accomplished otherwise, since nonneoplastic cells injected subcutaneously or intramuscularly are often difficult to retrieve. We believe that this new *in vivo* culture approach will effectively complement the existing *in vitro* culture systems used in studying the process of epithelial carcinogenesis.

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Pathological Changes Induced in Respiratory Tract Mucosa by Polycyclic Hydrocarbons of Differing Carcinogenic Activity*

D. C. Topping,† B. C. Pal, D. H. Martin, F. R. Nelson, and P. Nettesheim†

Seven aromatic polycyclic hydrocarbons (PCH) were investigated for their toxic effects on respiratory mucosa: benzo[*e*]pyrene (BeP), pyrene, anthracene, benz[*a*]anthracene (BaA), dibenz[*a,c*]anthracene (DBacA), benzo[*a*]pyrene (BaP), and dimethylbenz[*a*]anthracene (DMBA). The compounds were chosen because they comprise a spectrum of PCH ranging from noncarcinogens, to initiators, to weak and strong carcinogens. All of them except DMBA are environmentally relevant chemicals. The chemicals were tested over an 8-week period. Heterotopic tracheal transplants were continuously exposed, and the histopathological effects induced by the various PCH were periodically assessed in a semiquantitative fashion. All PCH exhibited varying degrees of toxicity for respiratory epithelium and submocosa. BeP clearly showed the least toxicity followed by pyrene and anthracene. BaA and DBacA caused marked epithelial and submucosal changes. In addition to epithelial hyperplasia, undifferentiated epithelium and squamous metaplasia developed. Marked mononuclear infiltration occurred in the subepithelial connective tissue. With BaP the epithelial and submucosal changes were similar but were much stronger. DMBA was the most toxic substance, causing epithelial necrosis followed by generalized keratinizing squamous metaplasia; the subepithelial changes consisted of an early acellular exudate and later (at 8 weeks) marked condensation and hyalinization of the lamina propria. The toxic response pattern of the tracheal mucosa to carcinogenic agents was characterized by the chronicity of epithelial and connective tissue damage, as opposed to the short-lived hyperplastic and inflammatory response elicited by the noncarcinogens and weak initiators.

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Toxic and Tumorigenic Effects of Asbestos on Tracheal Mucosa*

Douglas C. Topping, Paul Nettesheim, and Donald H. Martin

The purpose of the studies was to examine the acute and chronic effects of asbestos on the mucosa of conducting airways. Heterotopic tracheal grafts were used as the experimental model. In the system employed, the test substance was introduced into the airway lumen and kept in intimate contact with the respiratory tract mucosa for weeks or months. UICC chrysotile A and crocidolite asbestos caused marked acute changes of the tracheal mucosa: epithelial hyperplasia, hypersecretion of mucus, and submucosal inflammation. Particularly with chrysotile, the hypersecretory state and goblet cell hyperplasia persisted for many months, resulting in a marked distension of the tracheal grafts. Other manifestations of chronic asbestos toxicity were focal squamous metaplasias, epithelial erosions, and connective tissue changes involving the tracheal submucosa and adventitia, as well as the host subcutaneous tissues. The connective tissue changes were characterized by formation of granulomas, often with necrotic centers.

In a 29-month tumor-induction study carried out with chrysotile A, the total tumor incidence in 40 tracheal grafts was 40%; this included two squamous cell carcinomas (5%) and 14 sarcomas (35%), as compared with no carcinomas and 14% sarcomas in 148 historical controls. Thus our studies show that besides being a severe irritant for respiratory tract mucosa, chrysotile A also is a sarcomagenic and weakly carcinogenic agent for respiratory tract mucosa.

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Tumor Induction in the Trachea of Hamsters with *N*-nitroso-*N*-methylurea*

Tsutomu Yarita,† Paul Nettesheim, and Mary Lou Williams

Experiments were conducted to study the tumor response of hamster tracheas to *N*-nitroso-*N*-methylurea. Tracheas were exposed repeatedly using a tracheal catheter. A total of 10–30 exposures were given over a period of 5–20 weeks. The carcinoma incidence (including carcinoma *in situ*) was 0, 42, 67, 88, and 94% for 10, 15, 20, 25, and 30 twice-weekly exposures respectively. With 10 exposures, 2 out of 12 hamsters developed benign tracheal tumors. Mean tumor induction time decreased with increasing exposure frequency from 50 weeks with 10–15 exposures to 28 weeks with 25–30 exposures. The major histological types of invasive carcinomas observed were epidermoid carcinomas (54%), anaplastic large cell and small cell carcinomas (26%), adeno carcinomas (13%), and combined epidermoid-adeno carcinomas (7%). Sacrifice studies revealed that with 10–20 twice-weekly exposures only metaplastic lesions with varying degrees of cellular atypia are present at the time of the last exposure. Neoplastic lesions develop in the subsequent exposure-free interval. The data suggest that this tracheal tumor induction system may be well suited for studying problems related to development and progression of neoplastic disease.

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†Postdoctoral investigator, subcontract 3322 from the Biology Division of Oak Ridge National Laboratory to the University of Tennessee.

Carcinogenicity of Nickel Subsulfide for Respiratory Tract Mucosa*

Tsutomu Yarita† and Paul Nettesheim

The carcinogenicity of nickel subsulfide, Ni_3S_2, for respiratory tract epithelium was studied in heterotopic tracheal transplants using doses of 1 and 3 mg of Ni_3S_2 per trachea. Chemical determinations indicated that Ni_3S_2 persisted in the tracheas for 7–9 months. Ni_3S_2 showed marked toxicity for mucociliary epithelium, resulting in widespread atrophy and focal epithelial necrosis during the first 2 months of exposure. The submucosa showed mononuclear infiltration and signs of fibroblastic and capillary proliferation.

Tumor studies indicated that Ni_3S_2 can induce carcinomas in tracheal epithelium. The carcinoma incidence was 10% at 1 mg and approximately 1.5% at 3 mg. The higher dose produced a 67% incidence of fibro- and myosarcomas. The data suggest that, compared to some carcinogenic polycyclic hydrocarbons, Ni_3S_2 may not be a strong carcinogen for the epithelium of conducting airways. The data are discussed in the light of other experimental studies as well as epidemiological findings on respiratory tract cancers in nickel workers.

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Effects of Carcinogen Dose on the Characteristics of the Tracheal Tumor Response Induced by *N*-nitroso-*N*-methylurea in Hamsters*

Tsutomu Yarita † and Paul Nettesheim

Tracheal tumors were induced by repeated intratracheal exposures to the carcinogen *N*-nitroso-*N*-methylurea (NMU) using a catheter system previously described. Carcinogen concentrations of 0.25, 0.50, and 1.0% were employed in 20 or 30 twice-weekly exposures. Most of the tumors developed in the mid-portion of the tracheas. Virtually all tumors developed *after* the end of the 10- to 15-week exposure period. Tumor incidence ranged from 20–94% with mean tumor induction times of 13–46 weeks, depending on NMU concentration and frequency of exposure (*i.e.*, dose). At lower doses, mostly noninvasive tumors were induced; at higher doses, mostly invasive carcinomas of various histological types (epidermoid, adeno, epidermoid-adeno, and anaplastic large and small cell carcinomas) were induced. Adenocarcinomas were most frequent at low NMU concentrations. The possible mechanisms of the carcinogen dose effects are discussed.

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Tumor Development in Organ Transplants Obtained from Carcinogen-Exposed Donors*

Tsutomu Yarita† and Paul Nettesheim

Rats were exposed intragastrically to two different dose levels of *N*-nitrosoheptamethyleneimine (NHMI), resulting in a cumulative dose of either 225 or 450 mg/kg body weight. Tumor development was either followed *in situ* in the NHMI-exposed animals or in tracheas and esophagi from NHMI-exposed donors after grafting them to isogenic recipients. Tumor responses *in situ* and in organ grafts were compared. The results showed that the process of carcinogenesis is not disrupted by the transplantation procedure. The carcinogen dose-response relationship observed *in situ* was also seen in the transplanted organs. At the high carcinogen dose, the tumor incidence was 100% in *in situ* and transplanted esophagi, and 20% in tracheas *in situ* compared to 25% in tracheal transplants. At the low dose the tumor incidence was 36% in the esophagi *in situ* compared to 100% in transplanted esophagi, suggesting a greater sensitivity of the transplant system to detect the carcinogenicity of NHMI. The proportion of carcinomas to papillomas was markedly higher in transplanted esophagi. The tracheal tumor response at both NHMI dose levels showed the same trend but was too low to allow any firm conclusions.

The significance of these findings for studies of tumor development and progression and for the interpretation of whole-animal studies with "multifunctional" carcinogens is discussed.

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Tumor Development in Transplanted Organs from Nitrosamine-Exposed Donor Rats*

Tsutomu Yarita† and Paul Nettesheim

Abstract not available.

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Regulation of Thymic and Lympho-Hemopoietic Precursors in Bone Marrow

Joan Wright Goodman,* Robert Chervenak,† Sarah Garner Shinpock, Sandra J. Suppes,† and James R. Dasch§

Isogenic bone marrow (BM) from T-cell-deficient as well as control mice was injected into lethally irradiated (800–900 R) mice, and its capacity to promote regeneration of thymus, splenic lymphocytes, and marrow CFU-s populations was evaluated. Marrow was tested from the following doners: (*a*) normal untreated, (*b*) anti-Thy 1.2 treated, (*c*) isogenic chimeric, and (*d*) TIR (from doners that had been thymectomized as adults, lethally irradiated, and reconstituted with $\alpha\theta$ BM. It was found that recipients of TIRBM differed significantly from those of other groups with respect to recovery of thymus weight, lipopolysaccharide responsiveness, and CFU-s content. These findings are consistent with the interpretation that there exists a BM regulatory mechanism that governs the rate of differentiation of pluripotential stem cells to committed progenitors such as pre-T or pre-B cells. The data further imply that this regulation is effected at least in part by a thymus-dependent cell.

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In Vivo Cooperation of Murine Lymphocytes Sharing One Haplotype of the Transplantation Genes: A Requirement for Tolerance of the Nonshared Haplotype

Matko Marušić * and Eugene H. Perkins

The ability of mouse thymus–dependent (T) and bone marrow–derived (B) lymphocytes to cooperate in the (humoral) rejection of the rat Yoshida ascites sarcoma (YAS) was investigated. Mice of two hybrid constitutions, which had one parent of the same strain and the other of a different strain, were used. T and B cells thus shared one haplotype of the transplantation genes, but the other haplotype was different so that allogeneic recognition could take place in both directions. To investigate the influence of allogeneic recognition on T-B cooperation, mice were also made mutually tolerant by establishing long-term radiation chimeras. Erythrocytes and spleen cells of the chimera were shown by serologic analysis to be donor-type. When the chimera was to serve as tumor host and B-cell source, it was thymectomized prior to irradiation and reconstitution with Thy 1.2 antisera and complement-treated bone marrow (TIR). The evidence of tolerance was markedly reduced anti-host-type reactivity in short-term *in vivo* [^{3}H]thymidine uptake studies. Successful cooperation, manifested by YAS rejection in TIR mice given splenic T cells, was seen (a) whenever the transferred T cells and the cells of the TIR recipient were syngeneic, and (b) when the T cells were nonsyngeneic with the cells in TIR recipients but were obtained from chimeric donors and injected

into chimeric recipients. Chimerism of only T-cell donors or TIR recipients was not sufficient for YAS rejection to be induced. The data suggest that the allogeneic recognition on either T or B lymphocytes may thwart otherwise successful cell cooperation.

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Cooperation of Murine Semiallogeneic T and B Lymphocytes in a Primary Immune Response *In Vivo*. The Role of the Environment in which Lymphocytes Differentiate

Matko Marušić*

Primary humoral immune response to rat Yoshida ascites sarcoma grown in mice was used to study mouse thymus–dependent (T) and bone marrow–derived (B) lymphocyte cooperation. It was shown that $B6D2F_1$ T lymphocytes that do not cooperate with parental B lymphocytes can help parental B lymphocytes from $B6 \rightarrow B6D2F_1$ radiation chimeras to reject the tumor. However, when the bone marrow cells from $B6 \rightarrow B6D2F_1$ chimeras were used to reconstitute parental B6 mice, it was found that these $B6 \rightarrow B6D2F_1 \rightarrow B6$ mice lost their tolerance to D2 transplantation antigens, and that their B lymphocytes were not able to cooperate with $B6D2F_1$ T lymphocytes. It appeared that the differentiation process that has rendered nonsyngeneic chimeric cells able to cooperate was independent of the exogenous antigen.

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Transfer of Immunity by Transfer of Bone Marrow Cells: T-Cell Dependency

Matko Marušić *

Thymectomized, lethally irradiated mice reconstituted with normal bone marrow cells succumbed when challenged intraperitoneally with rat Yoshida ascites sarcoma (YAS) cells 40 days after irradiation and reconstitution. In contrast, thymectomized irradiated mice reconstituted with bone marrow cells from YAS-immune donors rejected the subsequent tumor challenge. Pretreatment of the bone marrow cells from immune donors with anti-Thy 1.2 antiserum and complement completely abolished the transfer of anti-YAS resistance.

Bone marrow cells from donors thymectomized 2 months before immunization enabled almost all recipients to reject YAS, but bone marrow cells from donors thymectomized 8 months before immunization protected only 50% of the recipients. Further analysis showed that mice thymectomized 8 months before immunization failed to generate anti-YAS antibody response, whereas the antibody response of mice thymectomized 2 months before immunization did not differ from that of nonthymectomized age-matched control mice. The data suggest that the immune reaction of mice against xenogeneic YAS requires long-lived T_2 lymphocytes.

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Cellular and Humoral Mechanisms in the Immune Response of Mice to Ehrlich Ascites Tumor

Matko Marušić*

Normal mice immunized with irradiated Ehrlich ascites tumor (EAT) cells rejected the EAT challenge 2 weeks later. In contrast, injection of irradiated EAT cells did not induce antitumor immunity in T-cell-deficient [thymectomized, lethally irradiated, and bone marrow–reconstituted (TIR)] mice. However, when TIR mice were injected intravenously with thymus, lymph node, or spleen cells from normal syngeneic donors immediately following intraperitoneal injection of irradiated EAT cells, they rejected the subsequent tumor challenge. The induction of immunity was shown to be T-cell dependent. This protocol appeared suitable for investigating the antitumor reaction of lymphoid cells from tumor-bearing mice and also for studying both the cellular and humoral components of the antitumor immune reaction. It was shown that spleen cells from EAT-bearing mice, which could not inhibit EAT growth when admixed with tumor cells before injection into normal recipients, were able to induce anti-EAT immunity in TIR mice when given immediately after irradiated tumor cells. To understand better the mechanism by which the transferred lymphoid cells induce anti-EAT immunity in TIR mice, spleen cells and serum from EAT-immune mice were tested in a series of experiments. No anti-EAT immunity could be demonstrated *in vitro*. In contrast, spleen cells from EAT-immune mice inhibited EAT growth when admixed with tumor cells prior to injection into normal recipients but had no effect on progressive tumor growth when given intravenously. Immune serum inhibited intraperitoneal EAT growth when given either intraperitoneally or intravenously. Inhibition of EAT growth by admixed spleen cells was shown to be T-cell dependent, but the activity of the immune serum appeared to be T-cell independent. The data indicate that the T lymphocytes are required only in the induction phase of the immune response of mice against EAT, while the efferent phase of the response is accomplished by serum antibodies, perhaps through an interaction with host macrophages.

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Transfer of Immunity by Transfer of Bone Marrow Cells: A Requirement for T Lymphocytes and Sensitivity to Cyclophosphamide

Matko Marušić*

Thymectomized, lethally irradiated mice reconstituted with bone marrow cells (TIR mice) from normal donors succumbed when challenged intraperitoneally with xenogeneic (rat) Yoshida ascites sarcoma (YAS) 1 month after irradiation and reconstitution. YAS rejection and production of anti-YAS antibodies was induced in these mice by a single intravenous injection of normal syngeneic spleen cells given 1 day after the tumor. Purified splenic T lymphocytes also induced YAS rejection in TIR mice, but splenic B lymphocytes did not affect a progressive tumor growth. Tumor challenge was also rejected in TIR mice that had been reconstituted with bone marrow cells from YAS-immune donors. These TIR mice did not have antitumor antibodies in their sera between reconstitution and YAS challenge but showed a high anti-YAS serum antibody titer after YAS challenge and rejection. Immunofluorescence staining did not reveal any dramatic differences in the content of T and B lymphocytes in the spleens and bone marrow between TIR mice reconstituted with bone marrow cells from normal donors and those reconstituted with bone marrow cells from the YAS-immune donors. Transfer of anti-YAS resistance was abolished when the bone marrow cells from immune donors were pretreated with anti-Thy 1.2 antiserum and complement, or when bone marrow donors were injected with cyclophosphamide 1 day after immunization. Cyclophosphamide was also shown to induce strong and specific suppression of anti-YAS antibody production in normal mice.

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Stimulation of Mouse Megakaryocyte Endomitosis by Plasma from Thrombocytopenic Rats*

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Injection of plasma from thrombocytopenic donor rats resulted in an increase in the endomitotic index of megakaryocytes of recipient mice 32 hr 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 TSF occurs via stimulation of megakaryocytopoiesis.

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A Positive Correlation Between Declining Immune Competence and Early Mortality Associated with Diethylnitrosamine Carcinogenesis in Aging Mice

Eugene H. Perkins, Neil K. Clapp, Lucia H. Cacheiro, Paul L. Glover, and William C. Klima

The effect of age (2.5, 9.5, and 17 months) at time of treatment upon diethylnitrosamine (DEN) carcinogenesis and immune competence has been assessed in female BALB/c mice. Median times of death were 193, 168, and 125 days, respectively, after termination of DEN treatment. Immune competence as a measure by both cell-mediated and humoral immune parameters immediately after DEN treatment was not significantly different among treated and age-matched nontreated control animals. In contrast, a significant age-related decline in immune competence was seen in both DEN-treated and nontreated controls, thereby demonstrating a direct and positive correlation between the natural age-related decrease in immune competence and cancer-induced advanced mortality.

Changes in Hepatic Levels of Tyrosine Aminotransferase Messenger RNA During Induction by Hydrocortisone*

Joanne M. Nickol,† Kai-Lin Lee, and Francis T. Kenney

Messenger RNA specific for tyrosine aminotransferase was quantitated by microinjection into oocytes of *Xenopus laevis*. The heterologously translated enzyme was identified by specific immunoprecipitation and found to be identical to authentic aminotransferase by several criteria. The level of functional message present in rat liver increases during hydrocortisone induction, and this increase is directly proportional to the increased rate of synthesis of the enzyme. Kinetic analysis of the changes in tyrosine aminotransferase mRNA levels during induction and withdrawal indicates that the steroid does not affect the stability of the message, which has a half-life of about 1.2 hr. Hydrocortisone, therefore, acts to increase the rate of synthesis of the specific messenger by stimulating either its transcription or processing to functional mRNA.

*Research supported jointly by Training Grant 5-T01-CA05296 from the National Institutes of Health and the Division of Biomedical and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with Union Carbide Corporation.

†Present address: Laboratory for Molecular Biology, National Institute of Arthritis, Metabolism, and Digestive Diseases, Bethesda, Maryland 20018.

Multiple Hormonal Control of Enzyme Synthesis in Liver and Hepatoma Cells

Francis T. Kenney, Kai-Lin Lee, Nicholas Pomato,* and Joanne M. Nickol†

Abstract not available.

*Postdoctoral investigator, Training Grant 5-T01-CA05296 from the National Institutes of Health. Present address: Simpson Memorial Research Institute, University of Michigan Medical School, Ann Arbor.

†Predoctoral investigator, Training Grant 5-T01-CA05296 from the National Institutes of Health. Present address: Laboratory for Molecular Biology, National Institute of Arthritis, Metabolsim, and Digestive Diseases, Bethesda, Maryland 20014.

Synthesis and Secretion of Transferrin by Cultured Mouse Hepatoma Cells

John Papaconstantinou, Robert E. Hill,* William H. Gibson,† and Edith Y. Rao

The mouse hepatoma cell (Hepa-1) in tissue culture has been shown to synthesize and secrete three electrophoretically distinct transferrins. Each of these forms of transferrin has a molecular weight of 77,000, as determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The concentration of each form is indicated by its staining intensity, which is highest in the form with the fastest mobility and lowest in the form with the slowest mobility. The relative rate of transferrin synthesis has been determined in log-phase and stationary-phase cells; the data indicate that the relative rate of synthesis increases twofold in stationary-phase cells. When the incorporation of [^{3}H]leucine into transferrin reaches steady state, the rate of secretion is equal to the rate of synthesis; the rate of secretion also increases twofold in stationary-phase cells. Our studies also show that transferrin synthesis accounts for 0.98% of the total protein synthesis in log-phase cells and for 1.8% in stationary-phase cells. This is the level of synthesis that has been determined by *in vivo* studies. We conclude that after continuous culture for several years these hepatoma cells have maintained one of the characteristics of the differentiated liver cell – namely, the ability to synthesize and secrete transferrin.

*Predoctoral investigator, Grant GM 1974 from the National Institute of General Medical Sciences.

†Supported by American Cancer Society Grant PF-1158.

Inhibition of Globin Translation by Glucocorticoids in Friend Leukemia Cells

John Papaconstantinou, Patrick R. McClintock,* Jeffrey P. Rabek,† and Edith Y. Rao

The response of Friend leukemia cells to steroid and polypeptide hormones was studied in cultures with and without dimethyl sulfoxide. With erythropoietin and etiocholanolone, hormones that are known to stimulate erythroid differentiation *in vivo*, no effect was observed on the ability of the cells to synthesize hemoglobin. Two glucocorticoids, hydrocortisone and dexamethasone, at concentrations of 10^{-5} to 10^{-8} *M*, proved to be potent inhibitors of the dimethyl sulfoxide–induced synthesis of hemoglobin, but they did not reduce cell growth or viability. DNA–RNA hybridization studies using globin cDNA indicated that globin mRNA accumulates in the presence of these hormones, and polyacrylamide gel electrophoresis showed that globin synthesis does not occur. We conclude that hydrocortisone and dexamethasone inhibit the translation of globin mRNA.

*Predoctoral investigator, Grant GM 1974 from the National Institute of General Medical Sciences. Present address: National Institute of Dental Research, Bethesda, Maryland 20014.

†Postdoctoral investigator, subcontract 3322 from the Biology Division of Oak Ridge National Laboratory to the University of Tennessee.

Synthesis and Secretion of Albumin by Cultured Mouse Hepatoma Cells

Barry E. Ledford,* Curtis L. Parker,† and John Papaconstantinou

Cultured mouse hepatoma cells (Hepa) derived from the solid tumor BW7756 have been shown to synthesize and secrete serum albumin. The relative rate of albumin synthesis has been determined in log- and stationary-phase cells; the data indicate that the relative rate of synthesis increases twofold in stationary-phase cells. When the incorporation of [^{3}H]leucine into albumin reaches steady state, the rate of secretion is linear and is equal to the rate of synthesis; the rate of secretion also increases twofold in stationary-phase cells. Our studies show that albumin synthesis accounts for 4.4% of the total protein synthesized in log-phase cells and 9.25% of that synthesized in stationary-phase cells. This is the level of synthesis observed to occur in rat liver *in vivo*. Although these cells maintain the *in vivo* level of albumin synthesis, our studies on albumin secretion have shown that a lag period of 30–35 min occurs before [^{3}H]albumin appears in the culture medium; this is considerably longer than the 15-min lag period that has been reported to occur in rat liver *in vivo*. We can offer no explanation for the significant difference in the lag periods. We conclude, however, that after continuous culture for several years these hepatoma cells have maintained some of the characteristics of the differentiated liver cell, one of which is their ability to synthesize and secrete serum albumin.

*Postdoctoral investigator, U.S. Public Health Service Fellowship GM 51857. Present address: Department of Biochemistry, Medical University of South Carolina, Charleston 29401.

†Present address: Department of Anatomy, Bowman-Gray School of Medicine, Winston-Salem, North Carolina 27103.

Mouse Albumin Messenger RNA in Liver and a Hepatoma Cell Line: Preparation of Complementary DNA from Purified Messenger RNA and Quantitation by Nucleic Acid Hybridization

Peter Brown* and John Papaconstantinou

Albumin, a major serum protein synthesized and secreted by the liver, is one of several serum proteins whose synthesis is regulated by hormonal and nutritional factors as well as during liver development. As a part of our studies on the regulation of serum protein synthesis, we have isolated mouse albumin mRNA by direct immunoprecipitation of albumin-synthesizing polysomes and oligo(dT)-cellulose chromatography of albumin polysomal RNA. This albumin mRNA was observed to sediment at about 17S, which corresponds to a molecular weight of approximately 6.5×10^5 daltons or 2000 nucleotides. Translation of this mRNA *in vitro* yielded a product that is immunoprecipitable with antimouse albumin and that showed a single radioactive peak having a molecular weight of 68,000 on sodium dodecyl sulfate–polyacrylamide gel electrophoresis. DNA, complementary to albumin mRNA, was synthesized with avian myeloblastosis virus RNA-dependent DNA polymerase. This complementary DNA (cDNA) was shown by alkaline sucrose density gradient sedimentation to have a molecular weight of 5.3×10^5 daltons, which is equivalent to 1740 nucleotides and represents approximately 87% of the total 17S mRNA. Hybridization of the cDNA to its template mRNA gave a $R_0t_{1/2}$ value of 2.3×10^{-3} mol•nucleotides•sec•liter^{-1} (in 0.5 *M* NaCl). The resultant cDNA-mRNA hybrid displayed a melting temperature (T_m) of 89°C when analyzed by thermal elution from a hydroxylapatite column, indicating a high degree of fidelity of the base pairings formed in this hybrid. Data from the hybridization analyses and cell-free translation studies indicate that the albumin mRNA is about 80–85% pure. Quantitation of albumin mRNA in total cytoplasmic RNA, by hybridization of cDNA under conditions of RNA excess, revealed that mouse liver contains about tenfold more albumin mRNA sequences than Hepa-2 cells, a permanent mouse hepatoma cell line that has maintained the capacity to synthesize albumin.

*Predoctoral investigator, Grant GM 1974 from the National Institute of General Medical Sciences.

Lead Metabolism in the Pigeon*

N. W. Revis and Judith Shaw

White Carneaux pigeons (*Columbiformes streptopelia*) were exposed to various concentrations of lead in drinking water to study the rate at which lead accumulates in blood and other tissues. The blood (11 mg lead/100 ml) and kidney (3.9 mg lead/g dry weight) showed the greatest increase in lead following 3 months of exposure to 200 ppm lead. Mice and rats exposed for 3 months to 200 ppm lead showed increases in both blood and kidney lead; however, these increases were not as great as observed in the pigeon. The kidney from mice or rats exposed to 200 ppm lead showed no histopathological alternations. However, the kidney from pigeons exposed a 200 ppm lead showed tubular degeneration, fibrosis, and acid-fast intranuclear inclusion bodies. Similar pathological alterations have been reported in man following chronic exposure to lead. These results strongly suggest that the pigeon may be an excellent animal model to study the yet unanswered questions related to chronic nephritis due to lead intoxication.

*Research sponsored jointly by the Environmental Protection Agency under Interagency Agreement 40-569-76 and the Division of Biomedical and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with Union Carbide Corporation.

Metabolism of Lipids in Experimental Hypertrophic Hearts of Rabbits

N. W. Revis and A. J. V. Cameron*

Cardiac hypertrophy was induced in rabbits by subcutaneous injection of thyroxine or isoprenaline or by surgically constricting the abdominal aorta. Alterations in lipid metabolism were observed in these hypertrophic hearts. Thyroxine or isoprenaline treatment increased the fatty acids in the serum and stimulated a marked increase in total lipids, triglycerides, and fatty acids in the hypertrophied myocardium. Coarctation of the aorta, in contrast, induced a significant increase in these lipids without significantly affecting serum-free fatty acids. Histochemical and morphological studies confirmed an increase in neutral lipids. It is suggested that the observed increase in fatty acids in the heart following thyroxine or isoprenaline treatment is related to the increase in serum-free fatty acids, which is followed by an increase in the removal by the heart of serum fatty acids. However, the amount that is removed exceeds the amount that is oxidized, which leads to an increase in lipid stores. The increase in lipid stores in the heart following coarctation of the aorta probably corresponds to the decrease in myocardial concentrations of carnitine. Serum lipid levels following coarctation were not significantly different from those of controls.

*Department of Cardiology, University of Glasgow, Glasgow, Scotland.

Association of Myocardial Cell Necrosis with Experimental Cardiac Hypertrophy

N. W. Revis and A. J. V. Cameron*

Cardiac hypertrophy was induced in rabbits by injecting thyroxine or isoprenaline, or by surgically constricting the abdominal aorta. An increase in heart weight was associated with a change in the ratios of bound to free forms of five lysosomal enzymes, a change in serum creatine phosphokinase and lactate dehydrogenase, and a change in the morphology of the myocardial cells. Isoprenaline treatment for 5 days induced a maximal change in heart weight, in the ratio of lysosomal enzymes, and in the serum enzymes. Thyroxine treatment was required for 15 days before maximal changes in heart weight, ratio, and serum enzymes were observed. In contrast, coarctation of the aorta caused a progressive change in heart weight, in the ratio of lysosomal enzymes, and in serum enzymes. These results suggest that necrosis of the myocardial cells does indeed accompany cardiac hypertrophy. It was further observed that autophagosomes, degenerating mitochondria in the myocardial cells during the induction of cardiac hypertrophy, and myofibril lysis were found, all of which confirm the suggestion of myocardial cell necrosis in the experimentally enlarged heart.

*Department of Cardiology, University of Glasgow, Glasgow, Scotland.

Glutathione Peroxidase Activity and Selenium Concentration in the Hearts of Doxorubicin-Treated Rabbits

N. W. Revis and Nives Marušić

Doxorubicin-treated cardiotoxicity was studied in the rabbit. Rabbits given intravenous injections of 1.5 mg of doxorubicin per kilogram of body weight (three times per week for 2 weeks) developed morphological and histological alterations in their hearts. In addition to these changes, both glutathione peroxidase activity and selenium concentration were significantly reduced in the hearts of the doxorubicin-treated rabbits. The decrease in glutathione peroxidase activity is probably related to the decrease in the concentration of selenium, since this enzyme requires selenium for activity and, furthermore, since *in vitro* studies failed to show any effect of doxorubicin on glutathione peroxidase. Although the mechanism responsible for the observed decrease in selenium is not known, an alteration in the selenium flux in the mycocardial cell may account for the observation.

The results of the present studies suggest that the observed increase in lipid hydroperoxides in the hearts of doxorubicin-treated animals may be the result of a decrease in glutathione peroxidase activity.

Metabolism of Selenium in Skeletal Muscle and Liver of Mice with Genetic Muscular Dystrophy

N. W. Revis, C. Y. Horton, and S. Curtis*

The specific activity of glutathione peroxidase and the concentration of selenium in muscles from mice with genetic muscular dystrophy were both significantly increased over those of control mice. Following pretreatment with sodium selenite (10 μg/100 g body weight) for 3 days, the specific activity of glutathione peroxidase and the concentration of selenium in muscle from the dystrophic mice were both significantly increased over values in muscle from control mice. When the dystrophic and control mice were fed a diet deficient in selenium, both the activity of glutathione peroxidase and the selenium concentration were significantly decreased in muscle from both groups. However, the magnitude of decrease was significantly greater in the muscle from the control mice than in the muscle from the dystrophic mice. These results suggest that muscle from dystrophic mice has a greater capacity for selenium absorption and retention than does muscle from control mice.

*Carnegie Corporation Summer Research Student from Knoxville College, Knoxville, Tennessee 37921.

A Possible Mechanism for Cadmium-Induced Hypertension in Rats

Nathaniel Revis

The mechanism of cadmium-induced hypertension was explored by measuring noradrenaline metabolism. Cadmium *in vitro* was shown to inhibit both monoamine oxidase and catechol-*O*-methyltransferase, the two enzymes which inactivate the neurotransmitters noradrenaline and adrenaline. However, rats that were injected or fed (via the drinking water) with cadmium showed that, among the tissues surveyed, these two enzymes were inhibited significantly only in the aorta. *In vitro*, cadmium was found to inhibit noradrenaline binding to membranes from the heart, lung, and kidney while stimulating binding to aortic membranes, which suggests that the effects may be specific. These results suggest that, in the aorta, cadmium may inhibit the two catabolic enzymes of noradrenaline, while at the same time stimulating noradrenaline binding. Thus the effects of noradrenaline on vascular smooth muscle would be increased as well as prolonged.

The Relationship Between Fibrosis and Lactate Dehydrogenase Isoenzymes in the Experimental Hypertrophic Heart of Rabbits

N. W. Revis and A. J. V. Cameron*

Cardiac hypertrophy was induced in rabbits by injecting either thyroxine or isoprenaline or by surgically constricting the abdominal aorta. An increase in heart weight was associated with a change in the lactate dehydrogenase (LDH) isoenzyme pattern and an increase in fibrosis (as measured by hydroxyproline concentrations). Isoprenaline treatment led to a moderate increase in heart weight, a marked decrease in the heart/skeletal muscle (H/M) subunit ratio of LDH, and a marked increase in hydroxyproline. Thyroxine treatment led to a small increase in both heart weight and hydroxyproline and a small decrease in the H/M subunit ratio. Coarctation of the aorta, in contrast, caused a marked increase in heart weight, a moderate decrease in H/M subunit ratio, and a moderate increase in hydroxyproline. These results suggest that the decrease in the H/M subunit ratio of LDH in the experimental hypertrophic heart reflects the extent of myocardial fibrosis, rather than changes within the hypertrophied myocardial cells.

*Department of Cardiology, University of Glasgow, Glasgow, Scotland.

Effects of Doxorubicin and Its Aglycone Metabolite on Calcium Sequestration by Rabbit Heart, Liver, and Kidney Mitochondria

N. W. Revis and Nives Marušić

The effects of doxorubicin and its aglycone metabolite on the $^{45}Ca^{2+}$-retention properties of mitochondrial fractions of the rabbit heart, liver, and kidney were investigated. High concentrations of doxorubicin (100 μM) inhibited mitochondrial retention of $^{45}Ca^{2+}$ in all the tissues studied. The aglycone metabolite (100 μM), contrary to doxorubicin, stimulated mitochondrial retention of $^{45}Ca^{2+}$ in all the tissues studied. Mitochondria from the heart were quantitatively more sensitive to doxorubicin and the aglycone metabolite than were mitochondria from the other two tissues. The consequences of these results are discussed in terms of the possible involvement of doxocubicin and its aglycone metabolite in the induction of mitochondrial swelling and mitochondrial inclusion bodies of heart mitochondria following doxorubicin treatment.

Sequestration of $^{45}Ca^{2+}$ by Mitochondria from Rabbit Heart, Liver, and Kidney After Doxorubicin or Digoxin/Doxorubicin Treatment

Nathaniel Revis and Nives Marušić

This study was conducted to determine if treatment of rabbits with doxorubicin (an anthracycline) would affect the ability of mitochondria isolated from heart, liver, and kidney to retain $^{45}Ca^{2+}$. Increases in mitochondrial retention of $^{45}Ca^{2+}$ by all of the tissues studied were observed, although only that from the heart showed a significant increase. The changes in $^{45}Ca^{2+}$ retention and morphology (i.e., increased mitochondrial swelling and intramitochondrial calcium phosphate crystals) of heart mitochondria from doxorubicin-treated rabbits suggest that the anthracycline directly or indirectly affects mitochondrial flux of calcium. That liver and kidney (as compared to heart) mitochondria are relatively insensitive to the effects of doxorubicin suggests a chemical difference in the mitochondria isolated from these tissues. Digoxin/doxorubicin treatment of rabbits, however, leads to a decrease in mitochondrial retention of $^{45}Ca^{2+}$, except for heart tissue, which again was significantly increased over the control. The effects of this treatment of the Na^+/K^+-activated ATPase of the heart, and on the accumulation of doxorubicin by the heart, were not significantly different from the control, suggesting that digoxin and doxorubicin do not compete for the same binding site.

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 assistantships or by one of the five federal training grants awarded to the School by (*a*) the National Cancer Institute, (*b*) the National Institute of General Medical Sciences, (*c*) the Department of Energy, (*d*) the National Institutes of Health in the form of a training grant in genetics, and (*e*) the National Institutes of Health in the form of a training grant in cellular and macromolecular sciences. The National Cancer Institute and National Institutes of Health training grants also carry support for postdoctoral training, and currently there are twelve postdoctoral fellows funded from these sources. An application for a training grant in environmental toxicology has recently been submitted to the National Institute of Environmental Health Sciences as has a training grant application in cardiovascular research to the National Institutes of Health. A training grant in environmental mutagenesis is in the process of being applied for. The School currently has 48 students working toward the Ph.D. degree and seven in the Master's program.

The fall quarter of 1978 marks the beginning of the School's thirteenth year. Drs. W. Edgar Barnett and R. Julian Preston are the Director and Associate Director respectively. The School also has five full-time faculty members: Drs. Donald E. Olins, Nathaniel W. Revis, Franklin D. Hamilton, Frank H. Gaertner, and Daniel Billen. Dr. E. A. Hiss has recently been appointed as Administrative Assistant to the Director, and Dr. Billen also serves as Director of The University of Tennessee Institute of Radiation Biology. 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.

At the end of the 1977–1978 academic year 50 Biomedical School students had been awarded the Ph.D. degree and five the M.S. degree. Students and their previous affiliations are listed below.

Laurel M. Adams, University of Tennessee
Kurt F. Amsler, University of Toronto
Barbara E. Armstead, Morgan State College
Richard J. Brake, University of Oklahoma
Alex J. Brown, Washington State University
Peter C. Brown, San Diego State University
Philip N. Bryan, University of Tennessee
James R. Burke, Manhattan College
Nelwyn T. Christie, Northeast Louisiana University
Shishir K. Das, Banaras Hindu University, India
Dale L. Dorsett, Wabash College
William G. Farmerie, Florida State University
James C. Fuscoe, III, San Diego State University
Theodore Gottlieb, Brooklyn College
Randolph J. Hellwig, Southern Illinois University
Robert E. Hill, University of Tennessee

Robert G. Holt, Florida A & M University
Sumin M. Huang, National Taiwan University
Norman J. Karin, Jr., University of New York at Fredonia
Richard C. Klann, University of Michigan
Charles M. Knowles, University of Tennessee
Chris R. LaBounty, State University of New York at Plattsburgh
Mary E. LaMarca, Oberlin College
Clinton D. Lothrop, University of Tennessee
Lloyd E. MacAskill, University of Wisconsin
Richard Machanoff, University of Tennessee
Ronald L. Manger, San Jose State University
Paula C. McGray, Michigan State University
Don Ray Miller, Texas Tech University
Claudia Moore, Western Carolina University
Magda H. Morales, University of Puerto Rico
Clarence C. Morse, University of Tennessee
Karen G. Nelson, Virginia Polytech University
Chin-Yih Ou, National Taiwan University
Robert K. Owenby, Carson-Newman College
Arthur E. Paton, San Diego State University
Stephanie T. Perry, East Central Oklahoma State University
Lewis R. Pollack, State University of New York at New Paltz
William F. Rall, State University of New York at New Paltz
Harry Ratrie, III, Virginia Military Institute
James C. Riddle, Denison University
Robin S. Rothrock, Hollins College
Thomas J. Savin, Fordham University
John V. Schloss, University of Tulsa
Ifen Grace Shiao, Florida State University
Elizabeth T. Snow, University of New Mexico
Rene Sotomayor, University of Chili
Gerald E. Spady, Washington State University
Kenneth R. Tindall, Montana State University
Frank Tsakeres, University of Tennessee
Dennis R. Voelker, Indiana University
Ann H. Weller, Ft. Lewis College
H. Steven Wiley, University of Tennessee
Donna S. Woodard, Stanford University
Cynthia P. Youmans, University of South Carolina

A list of graduates and their current affiliations follows.

Joseph C. Bagshaw, Wayne State University
Harvey L. Bank, Medical University of South Carolina
Randall Barton, University of Connecticut
K. L. Beattie, Baylor College of Medicine
Max Boling, Oak Ridge National Laboratory
Linda Borman, Oak Ridge Associated Universities
Emily Brake
Peter C. Brown, Stanford University
Steven N. Buhl, New York State University at Albany
Jose F. Calvino, Green Thumb Corporation, Florida

Eugene T. Chin, University of Texas
Maurice Cohen, Jr., California Institute of Technology
Dale DeHamer, University if Iowa
Gary R. Dunn, Harvard Medical School
Rosalie K. Elespuru, Frederick Cancer Research Center
Steve Fairfield
Raymond Fenwick, Texas Medical Center, Houston
Sarah A. Goodman, Emory University
Dale E. Graham, Purdue University
Brian Gray, University of California at Berkeley
James E. Hall
Russell Hand, Oak Ridge National Laboratory
Christie Holland, Worcester Institute for Experimental Biology
Suzanne Jackowski, University of Connecticut Health Center
Richard L. Krogsrud, Ontario Cancer Institute
J. Eugene LeClerc, Harvard Medical School
James L. Leef, Biomedical Research Institute, Maryland
Albert Li, University of New Mexico
Anna T. Li, University of New Mexico Medical Center
Janice Longstreth
Patrick McClintock, National Institute of Dental Research
Christine McDermott, Sloan-Kettering Institute for Cancer Research
JoAnne Nickol, National Institutes of Health
Ann C. Olson, Oak Ridge National Laboratory
Clifton Orr, University of Arkansas Medical Center
Jose R. Ortiz, University of Puerto Rico
Malcolm C. Paterson, Atomic Energy of Canada
Nelson H. Pazmino, Frederick Cancer Research Center
Gerald B. Price, Ontario Cancer Institute
Linda L. Pritchard, Groupe Hospitalier, Paul-Broussee, France
William R. Proctor, University of North Carolina
Richard J. Reynolds, Stanford University Medical Center
Charles O. Rock, Yale University
Kenneth J. Roozen, University of Alabama
Donald Salter, University of Illinois
John V. Schloss, University of Wisconsin-Madison
Paul B. Selby, Oak Ridge National Laboratory
Daniel M. Sheehan, National Center for Toxicological Research
Ronald J. Sheehy, Morehouse College
Juarine Stewart, University of Tennessee
Charles D. Stiles, Harvard Medical School
Gary Van Denbos, Northeastern Oklahoma State University
Anthony Vitto, University of California at San Diego
Cynthia Warner, Emory University
George R. Welch, University of Texas Medical School
Thomas Wilson, University of California at Irvine
Gail Wright

As of October 23, 1978, the Biology Division had 30 postdoctoral investigators (PDIs) who are supported through The University of Tennessee by a grant from the National Cancer Institute, a subcontract with Union Carbide Corporation, and a grant from the National Institutes of Health. Three additional PDIs are funded by outside support. A list of PDIs and their sponsors, grouped according to source of support, follows.

Supported by The University of Tennessee by a grant from the National Cancer Institute (Training Program – Carcinogenesis Research)

PDI	*Sponsor*
John H. Becker	J. S. Cook
Jeffrey S. Bohrman	T. J. Slaga
Lawrence W. Davenport	S. J. Kennel
Robert W. Eisinger	S. J. Kennel
Neil P. Johnson	R. O. Rahn
George H. Kidd	S. K. Niyogi
Mark D. Mamrack	S. M. Fischer
Kathleen R. Meyer	R. L. Ullrich
Cynthia J. Moore	J. K. Selkirk
Jackie L. William	J. Papaconstantinou

Supported by The University of Tennessee by subcontract with Union Carbide Corporation

PDI	*Sponsor*
Sankar P. Basu	J. R. Einstein
James A. Birchler	E. H. Grell
Andrew Butler	D. E. Olins
Gokul Das	S. K. Niyogi
Robert Foote	S. Mitra
Joseph Friedman	E. Huberman
David L. Hwang	D. E. Foard/W. K. Yang
Radhakrishman Iyer	T. J. Slaga
Pudur Jagadeeswaran	S. Mitra
Genevieve Jeannin	W. E. Barnett
Carol Jones	E. Huberman
James P. Kehrer	H. R. Witschi
Carolyn McKinley	R. F. Grell
James K.W. Mardian	D. E. Olins
Lee Opresko	R. A. Wallace
Patricia Pagni	R. K. Fujimura
Jeffrey Rabek	J. Papaconstantinou
Eng-Lay Tan	A. W. Hsie

Supported by The University of Tennessee by a grant from the National Institutes of Health (Training Program – Aging Research)

PDI	*Sponsor*
Ivan Collier	J. D. Regan
Lee W. Evans	J. Papaconstantinou

Supported by outside support

PDI	*Funding agency*	*Sponsor*
Terrence Donohue	National Cancer Institute	F. T. Kenney
John Frim	MRC of Canada	P. Mazur
Juan San Sebastian	Monsanto Toxicology Fund	A. W. Hsie

APPENDIX

Technical Meetings for Which Abstracts Were Submitted

October 1, 1977, through September 30, 1978

Fifth Annual Meeting of the Southeastern Cancer Research Association, Atlanta, Ga., Oct. 6–8, 1977

J. A. Young, T. K. Rao, T. J. Slaga, and J. L. Epler – Co-mutagenic properties of tumor-promoting agents in the *Salmonella typhimurium* microsomal activation system.

Annual Meeting of the Kentucky-Tennessee Branch of the American Society for Microbiology, Knoxville, Tenn., Oct. 21–23, 1977

J. S. Gill and H. I. Adler – Cell division factor of *Escherichia coli.*

J. A. Otten, R. W. Tennant, W. K. Yang, R. J. Rascati, and Arthur Brown – Transfer of *Fv-1* locus specific resistance to mouse tropic leukemia virus.

R. J. Rascati and R. W. Tennant – Induction of endogenous murine retraviruses: Mechanism of induction by hydroxyurea and related compounds.

Third Alumni Conference, Department of Biological Sciences, Florida State University, Tallahassee, Fla., Oct. 27–29, 1977

James D. Regan – DNA repair in mammalian cells: Effect of chemical carcinogens and Rauscher leukemia virus.

Symposium on Energy and Environmental Stress in Aquatic Systems, Augusta, Ga., Nov. 2–4, 1977

T. Wayne Schultz, J. N. Dumont, and Lola M. Kyte – Cytotoxicity of untreated coal conversion gasifier condensate.

29th Southeastern Regional Meeting, American Chemical Society, Tampa, Fla., Nov. 9–11, 1977

J. X. Khym and Elliot Volkin – Removal of interfering constituents prior to the determination of the cyclic nucleotides.

American Society for Cell Biology, San Diego, Calif., Nov. 15–18, 1977

John W. Bynum and Elliot Volkin – Characterization of chromatin-associated RNA synthesis in differentiating and neoplastic cells.

C. A. Chambers, M. Schell, and D. M. Skinner – The sequence of a satellite DNA whose separated strands do not associate with native DNAs.

N. T. Christie and D. M. Skinner – Interspersion of single copy DNA with sequences of satellite frequency.

F. H. Gaertner and G. E. Spady – The arom enzyme conjugate and proteases.

Christie A. Holland, Maria G. Schell, and Dorothy M. Skinner – The (G+C)-rich satellite of *G. lateralis:* An invertebrate satellite with a complex sequence and organization.

T. G. Hollinger, J. N. Dumont, and R. A. Wallace – Calcium-induced cortical granule breakdown in small oocytes from *Xenopus laevis.*

Robert G. Holt, Bam D. Mehrotra, and Franklin D. Hamilton – Studies on ribonucleotide reductase activity in *Pseudomonas stutzeri.*

Suzanne Jackowski and James N. Dumont – Time-dependent changes in surface structures of *in vivo* fertilized ova.

P. R. McClintock, J. P. Rabek, and John Papaconstantinou – Specific inhibition of globin mRNA translation by glucocorticoids.

J. F. McCarthy and D. M. Skinner – Molting and regeneration in crabs: Regulation of ecdysone titers during an interrupted proecdysis.

J. P. O'Neill, J. C. Riddle, and A. W. Hsie – Studies of G_1 phase of the cell cycle in Chinese hamster ovary cells in culture.

L. Opresko, H. S. Wiley, and R. A. Wallace – The origin of yolk-DNA in *Xenopus laevis.*

John Papaconstantinou, R. E. Hill, III, W. H. Gibson, Jr., and E. Y. Rao – Synthesis and secretion of transferrin by mouse hepatoma cells in culture.

Christina C. Scott, C. A. Heckman, Paul Nettesheim, and Fred Snyder – Lipid turnover in squamous-cell carcinoma cells and primary tracheal epithelial cells.

Kelly Selman and Robin A. Wallace – Maturation of Fundulus oocytes *in vivo* and *in vitro.*

30th Annual Scientific Meeting of the Gerontological Society, San Francisco, Calif., Nov. 18–22, 1977

G. P. Hirsch – Spontaneous mutations and evolutionary selection for long-lived genotypes in animals subject to predation.

Sumin M. Huang and Wen K. Yang – DNA polymerase activities in the spleens of young and old rats.

Diana M. Popp – Effect of busulfan on colony-forming units in the bone marrow of 4- and 18-month-old C3H mice.

New York Academy of Sciences Conference on Fibroblast Surface Protein, New York, N.Y., Nov. 30–Dec. 2, 1977

Morris D. Schneider and James N. Dumont – Burro aortic collagen: Location, source, and ultrastructural characteristics.

Symposium on The Syrian Hamster in Toxicology and Carcinogenesis Research, Bio-Research Institute, Inc., Cambridge, Mass., Nov. 30–Dec. 2, 1977

James K. Selkirk – Benzpyrene metabolism in hamster liver and in primary cell cultures.

Annual Meeting of the American Society of Hematology, San Diego, Calif., Dec. 3–6, 1977

T. T. Odell and D. A. Boran – Relation of severity of thrombocytopenia to stimulation of megakaryocytopoiesis.

Meeting on Methods for Carcinogenesis Tests at the Cellular Level and Their Evaluation for the Assessment of Occupational Cancer Hazards, Milan, Italy, Dec. 4–6, 1977

Eliezer Huberman – Cell-mediated mutagenesis and malignant transformation of mammalian cells in culture by chemical carcinogens.

International Symposium on Environmental Agents and Their Biological Effects, Hyderabad, India, Dec. 18–22, 1977

T. K. Rao, J. A. Young, A. A. Hardigree, and J. L. Epler – Mutagenicity of fractionated test material from the synthetic fuel technology with bacterial systems.

American Society of Zoologists and Society of Systematic Zoology, Toronto, Canada, Dec. 27–30, 1977

J. F. McCarthy and D. M. Skinner – Ecdysone metabolism during proecdysis in crabs.

Workshop on Higher Plant Systems as Monitors of Environmental Mutagens, Marineland, Fla., Jan. 16–18, 1978

J. L. Epler – Energy-related pollutants in the environment: The use of short-term tests for mutagenicity in the isolation and identification of biohazards.

ICN-UCLA Symposia on DNA Repair Mechanisms, Keystone, Colo., Feb. 19–24, 1978

William L. Carrier, David P. Smith, and James D. Regan – Pyrimidine dimer excision in human cells.

N. B. Kuemmerle and W. E. Masker – *In vitro* system for monitoring damage to and repair of T7 DNA.

Warren E. Masker and Nancy B. Kuemmerle – Studies on alternate pathways of dimer excision and repair resynthesis in *Escherichia coli.*

R. O. Rahn and R. S. Stafford – The generation of chain breaks and alkali-labile bonds upon photolysis of DNA containing iodinated cytosine residues.

Paul A. Swenson – Effects of a protease inhibitor on cessation of respiration and on viability of UV-irradiated *E. coli* B/r cells.

Raymond Waters and James D. Regan – Exchange between parental and daughter DNA strands in normal xeroderma pigmentosum and Bloom's syndrome fibroblasts.

Conference on Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures, Williamsburg, Va., Feb. 21–23, 1978

J. L. Epler, B. R. Clark, C.-h. Ho, M. R. Guerin, and T. K. Rao – Short-term bioassay of complex organic mixtures: Part II, Mutagenicity testing.

M. R. Guerin, B. R. Clark, C.-h. Ho, J. L. Epler, and T. K. Rao – Short-term bioassay of complex organic mixtures: Part I, Chemistry.

Abraham W. Hsie, 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 – Mutagenicity of carcinogens: Study of 101 individual agents and 3 subfractions of a crude synthetic oil in a quantitative mammalian cell gene mutation system.

Eliezer Huberman and Robert Langenbach – Mammalian cell mutagenesis.

Carroll E. Nix – The use of *Drosophila* in chemical mutagenicity testing.

Fourth Annual Hazardous Waste Research Symposium, San Antonio, Tex., Mar. 6–8, 1978

F. W. Larimer and J. L. Epler – Genetic toxicity testing of complex environmental effluents.

Environmental Mutagen Society Meeting, San Francisco, Calif., Mar. 9–13, 1978

N. L. A. Cacheiro and D. P. Manor – Causes of sterility in male mice derived from TEM treatment of different germ cell stages.

D. B. Couch, N. L. Forbes, and A. W. Hsie – Metal mutagenesis: Studies of the mutagenicity of manganous chloride and 14 other metallic compounds in the CHO/HGPRT assay.

R. B. Cumming – The potential for use of a centrifugal fast analyzer along with selective enzyme perturbing techniques as practical point mutational screens in mammals.

J. L. Epler, J. A. Young, T. K. Rao, M. R. Guerin, C.-h. Ho, and B. R. Clark – Isolation and identification of mutagenic components from synthetic fuels.

A. W. Hsie, J. P. O'Neill, D. B. Couch, J. R. San Sebastian, W. N. C. Sun, J. C. Fuscoe, J. C. Riddle, P. A. Brimer, R. Machanoff, N. L. Forbes, and M. H. Hsie – Mutagenicity of carcinogens: A preliminary validation of a quantitative mammalian cell mutation system, CHO/HGPRT, using 101 agents.

Maryala Krishna and Walderico M. Generoso – A procedure for preparing first-cleavage metaphase chromosomes from normally ovulated mouse eggs.

K. J. McDougall and J. F. Lemontt – Effects of spermine on forward mutation at the *CAN1* locus in *Saccharomyces cerevisiae.*

C. E. Nix, F. W. Larimer, T. K. Rao, A. A. Hardigree, B. Brewen, and J. L. Epler – Comparative mutagenicity of cyclic nitrosamines: Effects of methyl substitution and ring composition.

J. P. O'Neill, R. Machanoff, and A. W. Hsie – Factors affecting quantitative mutagenesis in the CHO/HGPRT system: Effects of phenotypic expression time, cell division, and cell density on mutant recovery.

T. K. Rao, F. W. Larimer, C. E. Nix, Della W. Ramey, and J. L. Epler – Comparative mutagenesis of cyclic nitrosamines: Effect of ring size.

L. B. Russell and C. S. Montgomery – Analysis of some of the conditions of the "spot test."

W. L. Russell – Specific-locus test for mutagenicity of benzo[*a*]pyrene in the mouse.

Gary A. Sega, R. E. Sotomayor, and J. G. Owens – Alkylation of germ cell DNA by methyl methanesulfonate in two mouse stocks and a comparison with ethyl methanesulfonate alkylation.

P. B. Selby – A new direct method of estimating genetic risk to humans from radiation and chemical mutagens.

J. A. Young, T. K. Rao, C. E. Weeks, T. J. Slaga, and J. L. Epler – Effect of the co-carcinogen benzo[*e*]pyrene on microsome mediated chemical mutagenesis in *Salmonella typhimurium*.

Joint Meeting of Biophysical Society and American Physical Society, Washington, D.C., Mar. 26–30, 1978

P. N. Bryan, E. B. Wright, M. H. Hsie, A. L. Olins, and D. E. Olins – Properties of inner histone-DNA complexes.

A. P. Butler, D. E. Olins. R. E. Harrington, and W. E. Hill – Salt and pH induced structural changes in the inner histones.

Neil P. Johnson, James D. Hoeschele, and Ronald O. Rahn – Comparative binding studies of *cis* and *trans*-dichlorodiammine ^{195}Pt(II) (DDP) to DNA.

A. Kootstra, T. J. Slaga, and D. E. Olins – Binding of benzo[*a*]pyrene diol epoxide to chromatin.

W. F. Rall, P. Mazur, and S. P. Leibo – Intracellular ice nucleation in 8-cell mouse embryos.

M. L. Villereal and J. S. Cook – Energy coupling for growth-associated enhancement of AIB accumulation in human fibroblasts.

M. Zama, D. E. Olins, B. Prescott, and G. J. Thomas, Jr. – Nucleosome conformations: pH and organic solvent effects.

American Cancer Society's 20th Seminar for Science Writers, Houston, Tex., Mar. 31–Apr. 4, 1978

Thomas J. Slaga – Anti-carcinogenesis: A rational approach to cancer prevention.

American Association for Cancer Research, Inc., Washington, D. C., Apr. 5–8, 1978

D. L. Berry, A. Viaje, S. M. Fischer, and T. J. Slaga – Kinetics of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) uptake and metabolism in newborn mouse epidermal cells in culture and adult cells in culture.

Neal K. Clapp, Lou C. Satterfield, Norman D. Bowles, and William C. Klima – Sex-specific protection by anti-oxidant butylated hydroxytoluene (BHT) upon 1,2 dimethylhydrazine (DMH) colon carcinogenesis in BALB/c mice.

C. A. Heckman, A. C. Olson, and A. C. Marchok – Morphological features altered with increasing oncogenic potential of respiratory tract epithelial cell lines.

Abraham W. Hsie, David B. Couch, Juan R. San Sebastian, William N. C. Sun, James C. Riddle, Patricia A. Brimer, Richard Machanoff, Nancy L. Forbes, Francis T. Kenney, and Mayphoon H. Hsie – Mutagenicity of carcinogens as determined in a quantitative mammalian cell mutation system, CHO/HGPRT: Study of 80 agents.

Stephen J. Kennel – Specific antibody to a murine alveolar cell carcinoma: Selection and purification from heterologous antiserum.

James O. Kiggans, Jr., David L. J. Hwang, and Wen K. Yang – *In vitro* binding of selective tRNA species to cellular and murine leukemia virus-coded RNA.

Robert Langenbach, Heather J. Freed, and Eliezer Huberman – Liver cell-mediated mutagenesis of mammalian cells with hepatocarcinogens.

J. Patrick O'Neill, James C. Fuscoe, and Abraham W. Hsie – Structure-activity relationships of antitumor agents in the CHO/HGPRT system: Cytotoxicity and mutagenicity of 8 ICR compounds.

Douglas C. Topping, Bimal Pal, and Paul Nettesheim – The interaction of benzo[*a*] pyrene and benzo[*e*] pyrene in respiratory tract carcinogenesis.

James K. Selkirk, Joellen Huisingh, and Ruggero Montesano – Benzo[*a*] pyrene metabolism (BP) in primary hepatocytes and normal and transformed rat epithelial cells.

Federation of American Societies for Experimental Biology, Atlantic City, N.J., Apr. 9–14, 1978

Jeffrey N. Clark and Ann C. Marchok – Mucin biosynthesis by rat tracheal explants: A model to study the biochemical and morphological effect of vitamin A.

Gerald P. Hirsch, Blaine S. Bradshaw, Mary W. Francis, Henry Francis, and Raymond A. Popp – Fidelity of bovine hemoglobin alpha chain synthesis assayed by cysteine complex formation with fluorescein mercuric acetate.

Ann C. Marchok and Paul Nettesheim – *In vitro* development of oncogenicity in cell lines established from tracheal epithelium preexposed *in vivo* to 7,12-dimethylbenz[*a*] anthracene (DMBA).

American Industrial Hygiene Conference, Los Angeles, Calif., May 7–12, 1978

T. K. Rao and J. L. Epler – Short-term mutagenicity testing related to oil shale technologies.

World Conference on Lung Cancer, Hilton Head Island, S.C., May 11–13, 1978

P. Nettesheim, A. Marchok, and P. Terzaghi – *In vivo* and *in vitro* studies on the progression of the neoplastic disease in respiratory tract epithelium.

Biologic Effects of Phorbol Esters in Cell Culture Systems Meeting, Cold Spring Harbor, N.Y., May 11–14, 1978

D. L. Berry, T. L. Sutton, A. Viaje, S. M. Fischer, and T. J. Slaga – Uptake and metabolic conversion of 12-*O*-tetradecanoylphorbol-13-acetate (TPA) by newborn and adult epidermal cells in culture.

Vernon E. Steele, Ann C. Marchok, and Paul Nettesheim – Growth promotion and tumor promotion by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in cultured respiratory epithelium.

26th Annual Meeting of the Radiation Research Society, Toronto, Canada, May 14–18, 1978

Howard I. Adler and James S. Gill – Stimulation of recovery and cell division by a particulate fraction of *Escherichia coli.*

Neal K. Clapp, Lou C. Satterfield, Norman D. Bowles, and William C. Klima – Selective effects of butylated hydroxytoluene (BHT) and diethylnitrosamine (DEN) on survival and carcinogenesis in mice when combined with X rays.

K. A. Davidson, J. M. Holland, J. W. Hall, and L. C. Gipson – Early effects of X irradiation on plasma estradiol-17β in two strains of mice.

R. J. M. Fry, E. J. Ainsworth, J. F. Thomson, F. S. Williamson, E. Staffeldt, and K. Allen – The prevalence of tumors in mice and the effects of radiation.

Eliezer Huberman – Mutagenesis and transformation.

R. L. Ullrich, M. C. Jernigan, and L. M. Adams – The induction of lung tumors in RFM mice after localized exposures to X rays or neutrons.

American Society for Microbiology, Las Vegas, Nev., May 14–19, 1978

G. Lavelle and L. Foote – Expression of baboon endogenous type C retrovirus by fetal, diploid baboon cells *in vitro.*

R. L. Schenley and P. A. Swenson – Loss of unaltered pyridine nucleotides from *E. coli* B/r cells after far UV irradiation.

A. S. Shetty and F. H. Gaertner – *Xanthomonas pruni,* an extant example of a major step in the evolution of the nicotinamide adenine dinucleotide biosynthetic pathway.

RNA Tumor Virus Meeting, Cold Spring Harbor, N.Y., May 24–28, 1978

W. K. Yang, D. L.-R. Hwang, F. C. Hartman, D. J. Price, J. O. Kiggans, C. D. Stringer, and D. M. Yang – Primer tRNA binding of cellular RNAs from retrovirus infected, transformed, and noninfected cells.

Brookhaven National Laboratory Symposium in Biology, Upton, N.Y., May 30–June 2, 1978

John V. Schloss, E. F. Phares, Mary V. Long, Claude D. Stringer, and Fred C. Hartman – Increased levels of ribulosebisphosphate carboxylase in *Rhodospirillum rubrum.*

Third National Conference on the Interagency/Environmental R & D Program, Washington, D.C., June 1, 1978

Michael D. Waters and James L. Epler – Status of bioscreening of emissions and effluents from energy technologies.

Genetics Society of America, Columbia, S.C., June 4–7, 1978

Edward G. Bernstine – The genetic control of mitochondrial malic enzyme expression in the mouse.

N. L. A. Cacheiro, Liane B. Russell, and Jean W. Bangham – A new mouse X-autosome translocation with nonrandom inactivation.

N. T. Christie and D. M. Skinner – The repetitive DNA of a primitive crab, *Geryon quinoquedens.*

R. B. Cumming – The genetics of formamidase 5 (brain formamidase) in the mouse.

Liane B. Russell, D. A. Carpenter, and G. D. Raymer – Tests for caffeine potentiation of radiation-induced chromosome anomalies.

W. L. Russell, R. B. Cumming, E. M. Kelly, and A. Lindenbaum – Plutonium-induced specific-locus mutations in mice.

American Society of Biological Chemists/Immunologists, Atlanta, Ga., June 4–9, 1978

B. S. Bradshaw, R. A. Popp, and G. P. Hirsch – Errors in protein synthesis detected by misincorporation of cysteine: Effect with age and X rays.

Kerry R. Foresman, David L. Wise, Joseph C. Daniel, Jr., and Raymond A. Popp – Sequence of blastokinin; A rabbit uterine protein.

R. K. Fujimura – Initiation of bacteriophage T5 DNA replication *in vitro*–analysis of DNA product.

Abraham W. Hsie, J. Patrick O'Neill, Juan R. San Sebastian, David B. Couch, James C. Fuscoe, William N. C. Sun, Patricia A. Brimer, Richard Machanoff, James C. Riddle, Nancy L. Forbes, and Mayphoon H. Hsie – Mutagenicity of carcinogens: Study of 101 agents in a quantitative mammalian cell mutation system, CHO/HGPRT.

David L. R. Hwang, James O. Kiggans, Jr., D. J. Price, D. M. Yang, and Wen K. Yang – *In vitro* hybridization of selective transfer RNA species with 18S and 28S ribosomal RNAs of various animal cells.

K.-L. Lee, R. E. Hill, and F. T. Kenney – Analyses of tyrosine aminotransferase regulation with antibodies to sequential and conformational determinants.

E. H. Perkins and L. H. Cacheiro – Failure to demonstrate immunodepression as a contributing component in radiation leukemogenesis of the RFM mouse.

S. T. Perry, K.-L. Lee, and F. T. Kenney – Reconstitution of aminotransferase holoenzymes *in vivo* and *in vitro:* Relationship to rates of metabolic turnover.

Diana M. Popp – Genetic control of IgA levels in mice.

Raymond A. Popp and Blaine S. Bradshaw – Altered concentrations of β-chain polypeptides in hemoglobins of α-thalassemic mice.

John V. Schloss, I. Lucile Norton, Claude D. Stringer, and Fred C. Hartman – Inactivation of spinach ribulosebisphosphate carboxylase/oxygenase by modification of arginyl residues.

Gerald E. Spady and Frank H. Gaertner – Evidence for at least 25 different proteases in *Neurospora*.

Mayo Uziel and J. K. Selkirk – Nucleoside excretion in growing and resting cells.

Mary Ellen Whitson – Induced cytotoxicity in normal human lymphocytes cultured *in vitro* with human multiple myeloma cells.

John J. Yim, Daniel D. Crummett, and K. Bruce Jacobson – A new enzyme in pteridine metabolism that cleaves the side chain of dihydroneopterin triphosphate.

Conference on Pollutants and High Risk Groups, Amherst, Mass., June 5–6, 1978

Paul Nettesheim – Vitamin A deficiency and carcinogenesis.

29th Annual Meeting of the Tissue Culture Association, Inc., Denver, Colo., June 5–8, 1978

J. S. Bohrman, S. M. Fischer, D. L. Berry, A. Viaje, and T. J. Slaga – Enhanced DNA synthesis by prostaglandin F-2α in the presence of 12-*O*-tetradecanoyl-phorbol-13-acetate in newborn mouse epidermal cells.

T. Ho, S. C. Tipton, and J. L. Epler – Cytogenetic effects of M-phenylene diamine (MPDA) and methylene dianiline (MDA) on human leukocytes *in vitro*.

J. L. Huisingh, S. Nesnow, J. K. Selkirk, J. P. Inmon, H. Bergman, B. Russell, and M. D. Waters – Comparison of benzo[*a*]pyrene metabolite formation in liver microsomes and cultured primary rat liver parenchymal cells.

E. F. Oakberg – Dynamics of follicular growth and atresia in the mouse.

V. E. Steele, A. C. Marchok, and P. Nettesheim – Expression of oncogenicity in epithelial cell lines derived from tracheal explants exposed *in vitro* to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG).

Annual Meeting of the American Association for the Advancement of Science, Pacific Division, Seattle, Wash., June 13, 1978

J. DiGiovanni, D. L. Berry, T. J. Slaga, and M. R. Juchau – Effects of Aroclor 1254 and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on polycyclic aromatic hydrocarbon skin carcinogenesis.

New York Academy of Sciences Meeting, New York, N.Y., June 22–27, 1978

David L. Berry, Thomas J. Slaga, John DiGiovanni, and Mont R. Juchau – Studies with chlorinated dibenzo-*p*-dioxins, polybrominated biphenyls and polychlorinated biphenyls in a two-stage system of mouse skin tumorigenesis: Potent anti-carcinogenic effects.

Symposium on Health Effects of Alternate Energy Sources, 1978 Air Pollution Control Association National Meeting, Houston, Tex., June 25, 1978

J. L. Epler and M. R. Guerin – Mutagenic components of alternate energy sources.

9th International Conference on Yeast Genetics and Molecular Biology, Rochester, N.Y., June 26–30, 1978

Jeffrey F. Lemontt – Different patterns of nonsense suppression of the pleiotropic *umr7-1* allele in *Saccharomyces cerevisiae*.

Sims 1978 Research Application Conference on Environmental Health, Alta, Utah, June 26–30, 1978

J. L. Epler – Environmental mutagenesis of energy-related compounds.

Workshop on Neoplastic Transformation in Differentiated Epithelial Cells *In Vitro*, Imperial Cancer Research Fund Laboratories, London, England, June 29–30, 1978

Ann C. Marchok, Vernon E. Steele, and Paul Nettesheim – Attempts to promote and inhibit transformation of tracheal epithelium exposed *in vitro* to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG).

American Society of Pharmacology and Experimental Therapeutics and Society of Toxicology Joint Meeting, Houston, Tex., Aug. 13–17, 1978

Gerald M. Cohen, Michael C. MacLeod, and James K. Selkirk – Organic solvent-soluble and water-soluble metabolites of benzo[*a*]pyrene formed by hamster embryo fibroblasts.

14th International Congress of Genetics, Moscow, USSR, Aug. 21–30, 1978

R. F. Grell – A temperature-sensitive recombination mutant in *Drosophila melanogaster.*

A. W. Hsie, J. P. O'Neill, D. B. Couch, J. C. Riddle, J. R. San Sebastian, P. A. Brimer, R. Machanoff, and M. H. Hsie – Utilization of a quantitative mammalian cell mutation system, CHO/HGPRT, in experimental mutagenesis and genetic toxicology.

E. F. Oakberg – Relevance of radiation-induced mutation rates in mouse oocytes to estimation of genetic hazards in the human female.

P. B. Selby – Do some reciprocal translocations cause malformations in translocation carriers?

Cold Spring Harbor Symposium on Transfer RNA, Cold Spring Harbor, N.Y., Aug. 22–27, 1978

K. Bruce Jacobson – Correlation between the absence of a $tRNA^{Tyr}$ isoacceptor and the activity of a mutant form of tryptophan oxygenase in *Drosophila.*

Wen K. Yang and David L. Hwang – Binding activities of selective tRNA species to cellular retrovirus-specific and ribosomal RNAs.

Annual Meeting of the International Society of Experimental Hematology, Chicago, Ill., Aug. 27–31, 1978

J. W. Goodman, S. G. Shinpock, R. P. Chervenak, S. J. Suppes, and J. R. Dasch – Regenerative capacity of T-cell depleted bone marrow.

T. T. Odell, C. Shelton, and T. P. McDonald – Stimulation of megakaryocytopoiesis in mice by plasma of thrombocytopenic donor rats.

IIIrd International Conference on Differentiation, Minneapolis, Minn., Aug. 28–Sept. 1, 1978

Charles E. Weeks, Thomas J. Slaga, and Eliezer Huberman – Effects of α-methylornithine and putrescine on differentiation and proliferation in Friend erythroleukemic cells.

XII International Congress of Microbiology, Munich, Germany, Sept. 3–8, 1978

Peter Mazur – Fundamental aspects of the preservation of microorganisms by freezing and freeze-drying.

VI International Biophysics Congress, Kyoto, Japan, Sept. 3–9, 1978

R. O. Rahn and R. S. Stafford – Photolysis of iodinated cytosine in DNA.

176th National Meeting of the American Chemical Society, Miami Beach, Fla., Sept. 10–15, 1978

R. Iyer, L. L. Triplett, T. J. Slaga, and J. Papaconstantinou – Interaction of (±)benzo[*a*]pyrene-7β, 8α-diol-9α, 10α-epoxide with fractionated eukaryotic DNA.

Magda H. Morales, P. C. Brown, and John Papaconstantinou – Regulation of α-fetoprotein synthesis in cultured mouse hepatoma cells (HEPA-2).

H. P. Witschi and W. Haschek – Some problems in correlating molecular mechanisms and cell damage.

International Research Group on Carcinoembryonic Proteins, Marburg, Germany, Sept. 17–21, 1978

Magda H. Morales, P. C. Brown, and John Papaconstantinou – Regulation of α-fetoprotein synthesis in cultured mouse hepatoma cells (HEPA-2).

National Symposium on Wastewater Disinfection, Cincinnati, Ohio, Sept. 18–20, 1978

R. B. Cumming, L. R. Lewis, R. L. Jolley, and C. I. Mashni – Mutagenic activity of nonvolatile organics derived from treated and untreated wastewater effluents.

32nd Annual Meeting of the Society for General Physiologists, Woods Hole, Mass., Sept. 21–24, 1978

Lewis R. Pollack and John S. Cook – Characterization and regulation of Na, K-ATPase in HeLa cells.

VII International Symposium on the Chemistry and Biology of Pteridines, La Jolla, Calif., Sept. 25–28, 1978

Dale Dorsett, John J. Yim, and K. Bruce Jacobson – Biosynthesis of drosopterins in *Drosophila melanogaster.*

Invited and Contributed Presentations

October 1, 1977, through September 30, 1978

H. I. Adler

Health aspects of energy production, Department of Zoology, Ohio Wesleyan University, Delaware, Ohio, October 1977.

Bacterial cell division, Department of Zoology, Ohio Wesleyan University, Delaware, Ohio, October 1977.

Cell division in bacteria, Department of Biology, University of Houston, Houston, Tex., November 1977.

Cell division in bacteria, Department of Zoology, University of Texas, Austin, Tex., November 1977.

W. E. Barnett

Molecular biology and evolution of eukaryotic organelles, East Tennessee State University, Johnson City, Tenn., October 1977.

Molecular biology and evolution of eukaryotic organelles, Florida State University, Tallahassee, Fla., October 1977.

Molecular biology and evolution of eukaryotic organelles, University of Georgia, Athens, Ga., November 1977.

Molecular biology and evolution of eukaryotic organelles, University of South Carolina, Columbia, S.C., November 1977.

Molecular biology and evolution of eukaryotic organelles, Clemson University, Clemson, S.C., February 1978.

Oak Ridge Associated Universities Traveling Lecture Program: Molecular biology and evolution of eukaryotic organelles, Biochemistry Department, Louisiana State University, Baton Rouge, La., October 1977; University of North Carolina, Asheville, N.C., January 1978; Texas A & M University, College Station, Tex., April 1978; Department of Biology, Paul Quinn College, Waco, Tex., April 1978.

D. L. Berry

In vivo and *in vitro* carcinogenesis – a multistage approach to the mechanism of chemical carcinogenesis, University of California and Western Regional Research Center, Berkeley, Calif., December 1977.

Initiation and promotion – a mechanistic approach to chemical carcinogenesis, University of California, School of Public Health, Los Angeles, Calif., March 1978.

Chemical carcinogenesis – comparison of *in vivo* models with *in vitro* models and mutagenesis, Departments of Food Science and Microbiology, Clemson University, Clemson, S.C., March 1978.

12-*O*-tetradecanoylphorbol-13-acetate (TPA) – its uptake and metabolism in mouse skin and newborn and adult epidermal cells in culture, University of North Carolina, Chapel Hill, N.C., April 1978.

TPA and tumor promotion – early biochemical events in mouse epidermal cells, School of Public Health, Johns Hopkins University, Baltimore, Md., May 1978.

E. G. Bernstine

Biochemical genetics of mitochondrial malic enzyme in mouse brain, Department of Biochemical Sciences, Princeton University, Princeton, N.J. April 1978.

Regulation of mitochondrial malic enzyme in the mouse, Roche Institute of Molecular Biology, Nutley, N.J., April 1978.

W. E. Cohn

How RNA got that way: Contributions of ion-exchange chromatography to molecular biology, University of Arizona, Tucson, Ariz., August 1978.

J. S. Cook

Turnover of plasma membranes and ouabain-binding sites in HeLa cells, Department of Biology, Syracuse University, Syracuse, N.Y., October 1977.

Turnover of plasma membranes and ouabain-binding sites in HeLa cells, Department of Physiology, Duke University, Durham, N.C., April 1978.

Turnover of plasma membranes and ouabain-binding sites in HeLa cells, Department of Biological Sciences, University of Cincinnati, Cincinnati, Ohio, May 1978.

Turnover of plasma membranes and ouabain-binding sites in HeLa cells, Department of Pharmacology, College of Medicine, University of Cincinnati, Cincinnati, Ohio, May 1978.

Regulation of amino acid transport in cultured human cells, Department of Biochemistry, University of Tennessee, Knoxville, Tenn., June 1978.

Transport regulation and turnover of membrane proteins, Department of Physiology, University of Southern California, Los Angeles, Calif., September 1978.

Transport regulation and turnover of membrane proteins, Department of Cellular Immunology and Aging, Wadsworth VA Hospital, Los Angeles, Calif., September 1978.

R. B. Cumming

Mutagenicity and water chlorination: Prospect and perspective, Second Conference on the Environmental Impact of Water Chlorination, Gatlinburg, Tenn., October 1977.

Mutagenesis testing in intact mammals, Symposium on Environmental Carcinogenesis, Michigan State University, East Lansing, Mich., May 1978.

J. DiGiovanni

The effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and aroclor 1254 on 7,12-dimethylbenz[*a*]anthracene metabolism and tumor initiating activity in mouse skin, Proc. Am. Assoc. Cancer Res., April 1978 (coauthors T. J. Slaga, D. L. Berry, and M. R. Juchau).

J. N. Dumont

Fertilization, development and selenium toxicity in *Fundulus heteroclitus*, Bell Baruch Institute for Marine and Coastal Research, Georgetown, S.C., June 1978.

J. L. Epler

Short-term mutagenicity assays, Food Microbiology Research Conference, Chicago, Ill., November 1977.

Mutagenicity work with shale oil, Second DOE Meeting and Workshop on Oil Shale Environmental Research, Richland, Wash., November 1977.

Analysis of complex mixtures – synthetic fuels, Subcommittee on Environmental Mutagenesis, Bethesda, Md., January 1978.

Health effects work with coal conversion, Industrial Environmental Research Laboratory–Environmental Protection Agency Advisory Meeting, Research Triangle Park, N.C., February 1978.

Health effects research, ORNL Research Committee Meeting, Oak Ridge, Tenn., March 1978.

Isolation and identification of chemical mutagens in complex mixtures, Philip Morris Research Center, Richmond, Va., May 1978.

Interim report on mutagenicity assays with aqueous leachates of solid wastes, Office of Solid Waste, Environmental Protection Agency, Washington, D.C., May 1978.

Mutagenicity testing of energy-related compounds, SIMS Research Application Conference on Environmental Health, Alta, Utah, June 1978.

Genetic research, prepared for Presidential Visit, ORNL, Oak Ridge, Tenn., May 1978.

Health effects research on energy related technologies, ORNL Summer Seminar Series, Oak Ridge, Tenn., July 1978.

Biological effects of synthetic fuels, ORAU Training Division, Faculty Institute on Coal Production, Technology, and Utilization sponsored by DOE, August 1978.

Health effects of shale oil, Third DOE Meeting and Workshop on Oil Shale Environmental Research, Los Alamos, N.M., August 1978.

Organic/bioassay analysis, Electric Power Research Institute Source Assessment Meeting, Palo Alto, Calif., September 1978.

Hazardous or not? – Mutagenicity of energy related compounds, Seventh National Congress on Waste Management Technology, Energy and Resource Recovery, San Francisco, Calif., September 1978.

Integrated chemical and biological testing of synthetic oils and effluents: Part I: Short-term mutagenicity testing, Symposium on Potential Health and Environmental Effects of Synthetic Fossil Fuel Technologies, Gatlinburg, Tenn., September 1978 (coauthors C. E. Nix, T. Ho, F. W. Larimer, T. K. Rao).

S. M. Fischer

Regulation of cell division and differentiation in mouse and human epidermal cells in culture. XV International Congress of Dermatology, Mexico City, Mexico, October 1977 (coauthors T. J. Slaga and D. L. Berry).

Lymphocyte blastogenesis and AHH, Human AHH Workshop, Council for Tobacco Research, New York, N.Y., February 1978.

Inflammatory agents and tumor promotion, Cold Spring Harbor Laboratory Meeting on Phorbol Esters, Cold Spring Harbor, N.Y., May 1978.

Prostaglandins and tumor promotion, National Cancer Institute Workshop on Human Tissue Culture, Washington, D.C., September 1978.

R. J. M. Fry

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F. D. Hamilton

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F. C. Hartman

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C. A. Heckman

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G. P. Hirsch

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Oak Ridge Associated Universities Traveling Lecture Program: Theories of aging: Trying to understand why we grow old, Biology Department, Grambling State University, Grambling, La., October 1977; Science Programs, University of Houston at Clear Lake City, Clear Lake City, Tex., November 1977; Gerontology Program, School of Allied Health Sciences, University of Texas Health Science Center at Dallas, Dallas, Tex., January 1978; Biology Department, Texas A & M University, College Station, Tex., January 1978; Center for the Study of Aging, North Texas State University, Denton, Tex., January 1978; Division of Natural Science and Mathematics, Queens College, Charlotte, N.C., February 1978; Department of Behavorial and Natural Sciences, Sacred Heart College, Charlotte, N.C., February 1978; Biology and Chemistry Departments, Southwestern at Memphis, Memphis, Tenn., February 1978; Convocation Lecture, Illinois College, Jacksonville, Ill., March 1978; Department of Natural Sciences, Maryville College, St. Louis, Mo., March 1978; Sigma Xi Society, Lake Forest College, Lake Forest, Ill., March 1978; Biology Department, University of Richmond, Richmond, Va., April 1978.

J. M. Holland

Observational bias in interpretation of murine survival data, Statistical Laboratory, University of California at Berkeley, Berkeley, Calif., October 1977.

Survival and spontaneous disease incidence in germfree athymic versus normal inbred C3H mice, Jackson Memorial Laboratory, Bar Harbor, Maine, May 1978.

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A. W. Hsie

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Alteration of tyrosine tRNA in *Drosophila*, Department of Microbiology, University of Tennessee, Center for Health Sciences, Memphis, Tenn., May 1978.

Protein synthesis, Biology II class, Oak Ridge High School, Oak Ridge, Tenn., April 1978.

Mechanism of suppression in *Drosophila*, Department of Pathobiology, University of California at Irvine, Irvine, Calif., September 1978.

F. T. Kenney

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Seminars

The following seminars were given in the Biology Division by visiting scientists from research organizations in the United States and abroad during the period October 1, 1977, through September 30, 1978. In addition, there were many informal seminars at which Division and Biomedical Graduate School members spoke about their own research.

Speaker	Affiliation	Subject
Phillip M. Achey[a]	Department of Microbiology, University of Florida	Repair of dimers in fish DNA and relationship to tumor formation
E. T. Adams, Jr.	Texas A & M University	Self association of protein
W. N. Aldridge	Medical Research Council Laboratories, Carshalton, England	Present views on the mechanism of toxicity of anti-esterase compounds
Ralph B. Arlinghaus[a]	University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute	Biosynthesis of murine leukemia virus reverse transcriptase by way of a 200,000 molecular weight "gag pol" polyprotein
Anne P. Autor	Toxicology Center, University of Iowa	Biochemical morphological aspects of pulmonary oxygen toxicity
Alok Bandyopadhyay	Baltimore Cancer Research Center	Effect of virus-specific proteins on reverse transcriptase
C. L. Borders[b]	College of Wooster	Role of arginine at enzyme active sites
Michael R. Boyd	Clinical Pharmacology Branch, National Cancer Institute	Clara cells and mixed function oxidases
Edward Bresnick[a]	Department of Biochemistry, University of Vermont College of Medicine	Nuclear activation of polycyclic hydrocarbons
Bryn A. Bridges	University of Sussex, England	Recent studies in bacterial mutagenesis
Donald D. Brown	Carnegie Institution of Washington	Genetics by gene isolation: The dual 55 RNA gene system in *Xenopus*
Ailsa M. Campbell	Department of Biochemistry, University of Glasgow, Scotland	Chromatin superstructure
C. Thomas Caskey[c]	Baylor College of Medicine	Biochemical characterization of point mutations in HPRT
Peter Cerutti[d]	University of Florida	Excisability of arylation productions of guanine in human lung cells
C. Paul Chow	Drug Research Laboratories, Drug Directorate, Health and Welfare, Canada	Comparative aspects of lead- and rubidium-induced hyperactivity in the rat
Ernest H. Y. Chu	Department of Human Genetics, University of Michigan	Genetical research and current emphasis on science and education in China
		Inverse relationship between galactokinase activity and 2-deoxygalactose resistance in Chinese hamster ovary cells
James E. Cleaver	Laboratory of Radiobiology, University of California	Coupling of excision repair and post-replication repair in normal and excision-defective human cells
W. E. Cleland[e]	University of Wisconsin – Madison	Cobalt and chromium ATP complexes as substrates for hexokinase and glycerolkinase
Gerald M. Cohen[a]	Department of Biochemistry, University of Surrey, England	Metabolism of benzo[*a*]pyrene by respiratory tissues
Mildred Cohn[f]	University of Pennsylvania	[^{31}P]NMR studies of enzymatic reactions of nucleotides

Speaker	Affiliation	Subject
J. E. Dahlberg	University of Wisconsin Medical School	tRNAs from ribosomal RNA precursors in *Escherichia coli*
D. T. Denhardt	Department of Biochemistry, McGill University, Montreal, Canada	Studies on the replication of DNA in *Escherichia coli*
Udo H. Ehling	Gesellschaft fur Strahlen-und Umweltforschung, Munich, Germany	Sensitivity of mutagenicity tests in mice
A. Monaem El-Hawari	Midwest Research Institute	Role of hepatic mixed function oxidases in ANIT (Alpha-naphthylisothiocyanate)-induced liver injury
Donald M. Engelman	Department of Molecular Biophysics and Biochemistry, Yale University	3-D mapping of ribosomal proteins
Anthony J. Faras[a]	Department of Microbiology, University of Minnesota Medical School	The mechanism of Rous sarcoma virus proviral DNA synthesis
Walter Farkas	University of Tennessee Memorial Research Institute	Effects of lead on purine metabolism: Mechanism of saturnine gout
Philip Feigelson[a]	Cancer Research Center, Columbia University	Hormonal and developmental control of specific hepatic messenger RNA in normal and neoplastic tissues
G. Felsenfield	Laboratory of Molecular Biology, National Institute of Arthritis, Metabolism and Digestive Diseases, N.I.H.	The organization of chromatin proteins
R. A. Floyd[a]	Oklahoma Medical Research Foundation, Biomembrane Research Laboratory	Free radicals in carcinogenesis
Werner W. Franke	German Cancer Research Center, Heidelberg, Germany	On the structure of the eukaryote nucleus
Norbert E. Fusenig	Deutsches Krebsforschungszentrum Institut für Biochimie, Heidelberg, Germany	Epidermal cell differentiation and *in vitro* transformation
H. G. Gassen	Institute of Organic Chemistry, Darmstadt, West Germany	Induced structure transition in RNA
A. Gentil	Institut de Recherche Scientifique sur le Cancer, Paris, France	DNA repair in mammalian cells after treatment with polycyclic hydrocarbon derivatives
Otto Hagenbuchle[a]	Department of Molecular Biophysics and Biochemistry, Yale University	Conserved sequences at the 3′-terminus of eukaryotic ribosomal RNA
Ingo Hansmann	Institute of Human Genetics, University of Göttingen, West Germany	Characteristics of developmental failure of haploid mouse embryos
William Hauswirth[a]	University of Florida	Adeno-associated virus: Replication of linear DNA in eukaryotic cells
A. Wallace Hayes	Department of Pharmacology and Toxicology, University of Mississippi Medical Center	Mechanistic approaches to toxicology
Harold G. Hempling[f]	Department of Physiology, Medical University of South Carolina	Cell water and electrolytes during maturation and the cell cycle
Peter Hinkle	Cornell University	Reconstitution of membrane transport systems
Eric Holtzman[a]	Department of Biological Sciences, Columbia University	Membrane circulation in neurons and photoreceptors
B. L. Horecker	Roche Institute of Molecular Biology	Modification of fructose 1,6-bisphosphatase by lysosomal proteases
Jerard Hurwitz	Albert Einstein College of Medicine	Studies on DNA synthesis *in vitro*

Speaker	Affiliation	Subject
Clyde A. Hutchinson III[h]	University of North Carolina	Construction of specific mutant DNA sequences in phage ΦX174
Helmut Ippen	Hautklinik and Poliklinik Kliniken der Universitat Göttingen, West Germany	Light sensitive diseases
Nobuhiko Katunuma	Institute for Enzyme Research, Tokushima University School of Medicine, Japan	Intracellular serine proteases structure and biological function
J. Katz	University of Tennessee Medical Unit	Nucleoside-Q in tRNA
Brian Kilbey	University of Edinburgh, Scotland	Initiation of UV mutagenesis in yeast
Zores A. Medvedev[i]	National Institute for Medical Research, London, England	Effects of age on the fidelity of HI histone biosynthesis in mouse tissue
H. Paul Meloche	Papanicolaou Cancer Research Institute	Three-dimensional games with enzymes
Martin L. Meltz	Southwest Foundation for Research Education	Delayed reassociation of repair replication in unique DNA sequences after mutagen treatment with human cells
R. Montesano[a]	Unit of Chemical Carcinogenesis, International Agency for Research on Cancer World Health Organization, Lyon, France	Alkylation and carcinogenesis by *N*-nitroso compounds
B. E. Moseley	University of Edinburgh, Scotland	High-fidelity repair of DNA damaged by mutagens in *Micrococcus radiodurans*
Daniel W. Nebert	Developmental Pharmacology Branch, National Institutes of Health	Genetic differences in drug metabolism affecting individual risk for cancer
Siv Osterman-Golkar	Wallenberg Laboratory, University of Stockholm, Sweden	Hemoglobin as a dose monitor for chemical mutagens
D. Oxender[a]	University of Michigan	The role of tRNA regulation of leucine transport in *Escherichia coli*
R. Palmiter[a]	Department of Biochemistry, University of Washington	Hormonal regulation of egg white protein synthesis
Walter B. Panko[a]	Institute of Comparative Medicine, Texas A & M University/Baylor College of Medicine	Biochemical anomalies in human breast cancer
Harry Peck[j]	Department of Biochemistry, University of Georgia	Biochemistry and physiology of sulfate-reducing bacteria
Michael G. Rossmann[g]	Department of Biological Sciences, Purdue University	Convergent and divergent evolution in protein structure
		Structure of southern bean mosaic virus
Wolfram Saenger	Max-Planck-Institut, Göttingen, West Germany	Cyclodextrin as enzyme models
A. G. Searle	Medical Research Council, Harwell, England	Complementation problems with mouse translocations
Dale Sevier[a]	Bioscience Laboratories, Van Nuys, California	Clinical assays in cellular immunology
Charles J. Sherr	Viral Pathology Section, Laboratory of Viral Carcinogenesis, National Cancer Institute	Studies of type C viral integration site and sarcoma-specific antigen
Jo Simons	Department of Radiation Genetics and Chemical Mutagenesis, State University of Leiden, The Netherlands	Mutation research with cultured mammalian cells

Speaker	Affiliation	Subject
Kendric C. Smith[d]	Department of Radiology, School of Medicine, Stanford University	Multiple pathways of DNA repair and their role in mutagenesis
F. H. Sobels	Department of Radiation Genetics and Chemical Mutagenesis, State University of Leiden, The Netherlands	Problems with estimating risks from chemicals
Joseph Sperling[g]	The Weizmann Institute of Science, Rehovot, Israel	Photochemical cross-linking – A probe of protein-nucleic acid interaction
L. C. Stevens	The Jackson Laboratory	Teratogenesis and parthenogenesis
P. V. Sundaram	Max Planck Institut für Experimentelle Medizine, Göttingen, Germany	Use of nylon in immobilized enzymes and applications
Steve Thompson	Millipore Corporation	Ultra-filtration
James Trosko[a]	Department of Human Development, Michigan State University	Integrated theory of carcinogenesis: The role of mutations and epigenetic changes
K. Van Holde[g]	Oregon State University	Recent advances in the study of chromatin structure
Robert A. Weinberg[a]	Massachusetts Institute of Technology Center for Cancer Research	Forms of proviral DNA of murine leukemia viruses
Ken Wheeler	University of Rochester	*In vivo* repair of X-ray induced DNA damage in normal brain tumors
Meir Wilchek	The Weizmann Institute of Science, Rehovot, Israel	Affinity labeling of enzymes, antibodies, ribosomes, and cells
Jerry Williams[i]	Harvard School of Public Health	DNA repair and aging
Reen Wu[a]	National Institute of Environmental Health Sciences	Growth regulation of HeLa S-3 cells by epidermal growth
C. S. Yang[a]	Department of Biochemistry, New Jersey Medical School	The role of microsome and nuclei in the metabolic activation of chemical carcinogens
G. Zaccai	Institute Max von Laue – Paul Langevin, Grenoble, France	Some neutron scattering results on biological systems – Models and natural membranes and protein nucleic acid interactions

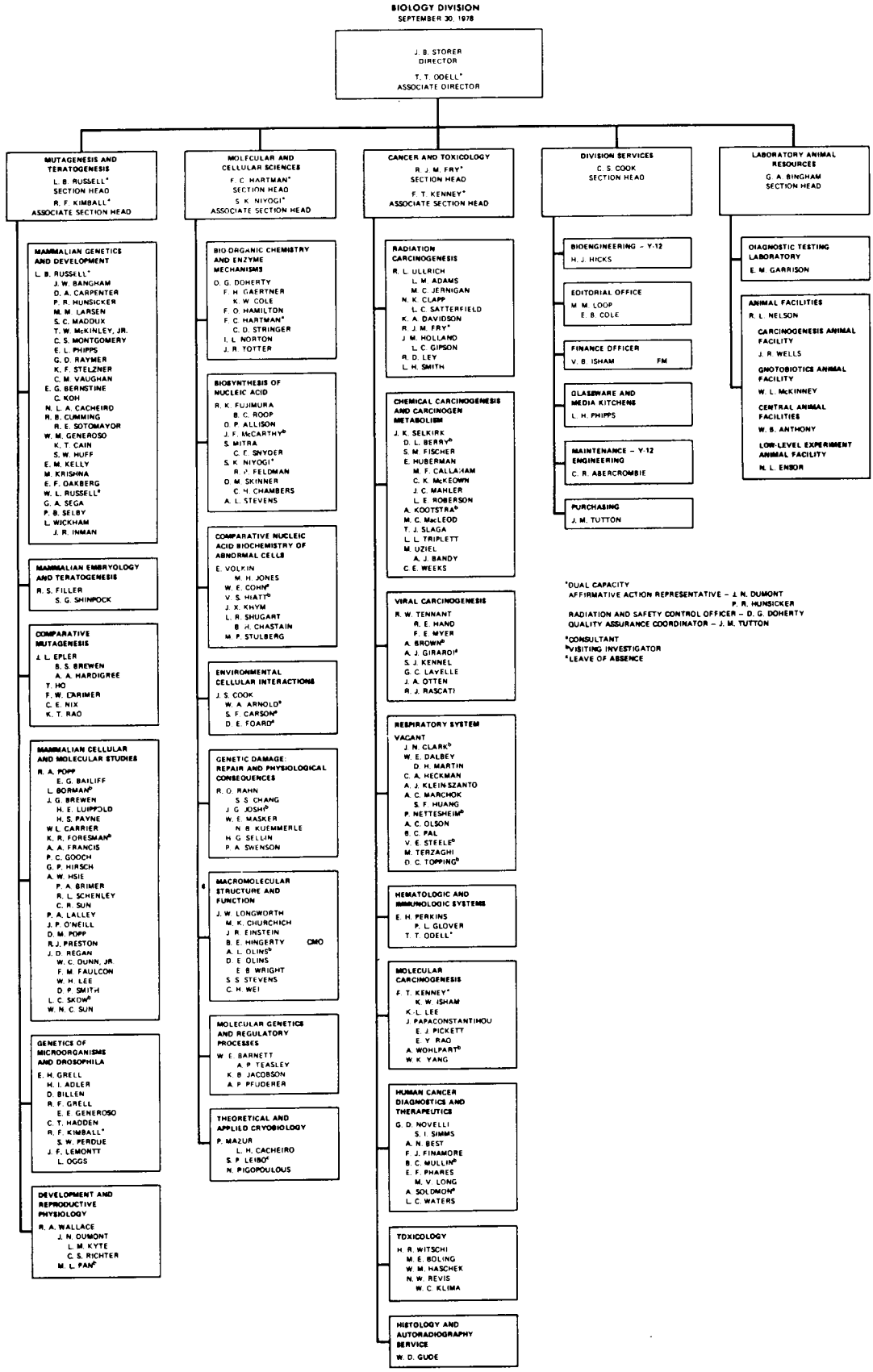
[a]Cancer Research Seminar.
[b]Biochemistry Journal Club.
[c]Genetics Seminar.
[d]Institute of Radiation Biology and University of Tennessee–Oak Ridge Graduate School of Biomedical Sciences.
[e]Biomedical Graduate School Student Seminar Series.
[f]Distinguished Lecturer Series.
[g]Molecular and Cellular Sciences Program Seminar Series.
[h]Genetics and Developmental Biology Seminar Series.
[i]Molecular Mechanisms of Aging Seminar Series.
[j]Biomedical Graduate School Distinguished Lecturer Series.

Annual Research Conferences

The thirtieth annual research conference of the Biology Division was held in Gatlinburg, Tennessee, March 28–31, 1977. The subject was Mechanisms of Tumor Promotion and Cocarcinogenesis, and T. J. Slaga and Paul Nettesheim were co-chairmen of the organizing committee. The proceedings of the meeting were published in 1978 by Raven Press as Volume 2 in their *Carcinogenesis – a Comprehensive Survey* series.

The 1978 symposium on Genetic Mosaics and Chimeras in Mammals was held in Gatlinburg, Tennessee, April 3–6, 1978. Liane B. Russell was chairman of the organizing committee, and the proceedings will be published in the near future by Plenum Press.

The 1979 meeting on The Scientific Basis of Toxicity Assessment is scheduled to be held in Gatlinburg, Tennessee, April 15–19, 1979. The symposium will address itself to problems and issues in toxicology, new outlooks on testing, mechanisms of toxicity, environmental transport problems, and the future of toxicology as a predictive science. Dr. Hanspeter R. Witschi is chairman of the organizing committee, and additional information on the meeting can be obtained by writing directly to Dr. Witschi.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