MECHANISMS OF CHEMICAL CARCINOGENESIS
Curtis Harris and Peter Cerutti, Organizers
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Plenary Sessions

Workshops and Poster Sessions

Carcinogen Metabolism and Carcinogen Interaction with Cellular Macromolecules

402 METABOLISM OF POLYNUCLEAR AROMATIC HYDROCARBONS, James K. Selkirk, Michael C. MacLeod, and Cynthia J. Moore, Biology Division, Oak Ridge National Laboratory and The University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences, Oak Ridge, TN 37830.

For some years it has been known that polynuclear aromatic hydrocarbons (PAH) are metabolized by mammalian cells to more polar and therefore readily excretable derivatives. This detoxification effort leads also to highly reactive intermediates which can form adducts with cellular macromolecules and thereby cause biological damage including cytotoxicity, mutagenesis, and malignant transformation. In order to understand and ultimately manipulate the events leading to the malignant state it is necessary to understand the complex balance of detoxification and activation reactions. A major conclusion of recent studies of the metabolism of PAH is that this balance is drastically perturbed in cell-free systems and that intact cells, tissues or animals are more appropriate systems of study. This is due not only to the absence of the conjugative mechanisms of secondary metabolism in disrupted cell systems, but also to observed differences in the regioselectivity of the primary metabolic enzymes between rat liver microsomes and cell culture systems. Using a number of cell culture systems, we have investigated the relationship between metabolism and carcinogenicity for selected PAH in the context of the bay-region theory. Using both rat liver microsomes and hamster embryo cell cultures, we found that benzo(e)pyrene [B(e)P] is metabolized at sites distal to the bay-region, and that little or no B(e)P-9,10-diol, the precursor to the bay-region diol-epoxide, is formed. The metabolic data thus provide an explanation for the lack of carcinogenicity of B(e)P. Such a simple correlation is not, however, observed for B(a)P. In this case the precursor to the bay-region diol-epoxide, B(a)P-7,8-diol, has been found to be formed in significant quantities in all cell culture systems examined, including those in which B(a)P is not normally carcinogenic (i.e., cells derived from liver). Furthermore, the metabolism of 2-OH-B(a)P, which is as carcinogenic as B(a)P on mouse skin, does not appear to favor formation of a bay-region diol-epoxide. Thus, an understanding of metabolic pathways in a given system is a necessary but not always sufficient condition for understanding carcinogenicity.

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403 METABOLISM OF 2-ACETYLAMINOFLUORENE (AAF) AND INTERACTION WITH CELLULAR MACROMOLECULES IN LIVER, Charles C. Irving, Veterans Administration Medical Center and Departments of Biochemistry and Urology, University of Tennessee Center for the Health Sciences, Memphis, TN, 38104

The metabolism of AAF has been studied extensively over the past 30 years, and these studies have contributed significantly to the substantial progress made in the field of chemical carcinogenesis. Despite this, we do not fully understand the biochemical mechanisms involved in the activation of AAF and related carcinogenic aromatic amines and amides. Several important findings and general concepts have emerged from these studies (reviewed in refs. 1 & 2). The aromatic amines and their N-acetyl derivatives are activated in vivo by multiple pathways. Differences in these metabolic pathways largely account for the differences in tissue and species susceptibilities to cancer induction. In rodent liver, in vivo, there is not a simple positive relationship between capacity to activate AAF to reactive intermediates that bind covalently to DNA and susceptibility to hepatocarcinogenesis. Further, the ability of liver to activate AAF in vivo cannot be correlated with the capacity of liver cell fractions to form mutagenic products in vitro. These findings should be kept in mind in the interpretation of data generated from in vitro mutagenesis studies with activation by liver cell fractions. Although metabolic activation represents an essential facet in the initiation of cancer by most chemicals, it is by no means a sufficient condition for the induction of cancer by these compounds. Rodent liver, for example, has a tremendous capacity to activate a variety of chemicals, but only a few of these will actually induce liver cancer in the normal adult rodent. Much more emphasis needs to be given to study of the additional factors involved in the carcinogenic process.

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404 ALKYLATION OF DNA AND TISSUE SPECIFICITY IN NITROSAMINE CARCINOGENESIS, R. Montesano
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The nitrosamines show a high degree of tissue and organ specificity in tumour induction and there is substantial evidence that the initiation of the carcinogenesis process by this group of carcinogens is linked to the metabolic competence of the target tissue or cell to convert these carcinogens into mutagenic metabolites and to the binding of these metabolites to cellular DNA (1, 2). Alkylation occurs in the DNA at the N-1, N-3, N-7 positions of adenine, N-3, N-7 and O^6 pf guanine, N-3 and O^2 of cytosine, and N-3, $O^4$and O^2 of thymine and the initial proportion of each DNA adduct depends upon the type of alkylating agent used (2). These various DNA adducts are lost from DNA *in vivo* by spontaneous base release and/or by specific DNA repair processes (3). *In vitro* (4) and *in vivo* (5) studies indicate that alkylation in the O^6 position of guanine is more critical than other DNA adducts in the mutagenesis and carcinogenesis induced by N-nitroso compounds. In particular tissues in which tumours more frequently occur after a pulse dose of nitrosamine are those in which the persistance of O^6-alkylguanine in DNA is the greatest; thus presumably resulting in an increased probability that a miscoding event (mutation) will take place during DNA synthesis. The rapid removal, as compared to extrahepatic tissues, of O^6-methylguanine from liver DNA of rats has been associated with the lack of tumours in this organ after a single dose of dimethylnitrosamine (DMN); however a significant incidence of liver tumours is observed if the same dose is given 24 hours after partial hepatectomy (6). Conversely tumours are induced by a similar dose of DMN in liver of hamsters, which has a low capacity to remove O^6-methylguanine from its DNA (7). From these data it also appears that the rate of 7-methylguanine in the liver or extrahepatic tissues is independent of the dose of DMN; whereas a more rapid loss of O^6-methylguanine from DNA occurs after a low dose of DMN. It has been shown that in rat liver the removal of O^6-methylguanine but not of other DNA adducts from DNA can be affected by pretreatment of the animals with N-nitrosocompounds (8, 9). The modulation of the DNA repair processes observed after a single dose or chronic treatment with nitrosamines is discussed in relation to the tissue specific carcinogenic effect of this group of carcinogens.

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405 EXCISABILITY AND PERSISTENCE OF CARCINOGEN INDUCED CHROMOSOMAL DAMAGE, Peter A. CERUTTI
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The concentration and distribution of persistent DNA lesions at the time of replication may represent a determining factor for the cytotoxic, mutagenic and transforming potency of a carcinogen. Persistence is determined by the constitutive or induced excisability of a specific lesion in relation to the proliferative capacity of a particular tissue. Nucleosomal- and higher order chromatin structure may impose constraints on lesion excisability. Changes in chromatin structure may enhance or suppress lesion excisability in different portions of the genome and thereby modulate the biological response to a carcinogen. Experimental support for these concepts is derived from studies with cultured human cells which were treated with Aflatoxin B_1, N-acetoxy-acetylaminofluorene or benzo(a)pyrene and its ultimate metabolites as DNA damaging agents. (Work supported by the Swiss NF and the ASFC).

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406 GENETIC DIFFERENCES IN DRUG METABOLISM AFFECTING INDIVIDUAL RISK OF MALIGNANCY, Daniel W. Nebert, Masahiko Negishi, Lynn W. Enquist, and David C. Swan, National Institutes of Health, Bethesda, MD 20205

The Ah locus represents a complex of genes controlling the induction of numerous drug-metabolizing enzyme "activities" by polycyclic aromatic compounds. The major regulatory gene product is a cystolic receptor, similar in many ways to the steroid receptors. Structural gene products include two distinctly different forms of cytochrome P-450, named arbitrarily P_1-450 and P-448. "Ah-nonresponsive" mice do not have detectable amounts of the receptor, and P_1-450 is therefore poorly induced following treatment with polycyclic aromatic chemicals. Allelic differences at the Ah locus have been shown to be associated in the mouse with increased individual risk for cancer, mutation, DNA binding of reactive metabolites, drug toxicity, birth defects, and enhanced detoxication. These differences appear to reflect increases or decreases in the steady-state level of reactive intermediates in target tissues--because of quantitative increases in P_1-450 and/or qualitative changes in the metabolite profile generated by newly induced P_1-450. The "Ah-responsive" individual who is at increased risk for cancer caused by subcutaneous, topical, or intratracheal polycyclic hydrocarbons is at decreased risk for toxicity of the bone marrow and leukemia caused by oral benzo[a]pyrene, (when compared with the genetically nonresponsive individual receiving the same dose of the same foreign chemical). In other words, tissue sites in direct contact with the carcinogen develop cancer more readily in responsive animals because of induced P_1-450; tissues in distant sites of the body may develop malignancy more readily in nonresponsive animals because more carcinogen reaches that tissue due to decreased P_1-450 induction in portal-of-entry tissues and therefore a decrease in detoxication. Hence, not only the dose but the route of administration, the timing of the dosage, and the site of the tumor or toxicity are all very important in the interpretation of data from carcinogenesis or toxicity experiments involving P_1-450 inducers such as polycyclic hydrocarbons.

We recently have isolated and characterized a cloned sequence of DNA associated with the murine Ah locus (most likely the P_1-450 structural gene). A second clone also is being characterized. In the next several years we hope to understand the genetic mechanism controlling the induction of drug-metabolizing enzymes--whether this be gene duplication, intragenic recombination, or some other mechanism. This information will provide valuable insight into evolutionary phylogeny, since P-450 is ubiquitous in certain bacteria and presumably all plants and animals. These cloned genes also may aid us in understanding the mechanism of chemical carcinogenesis. Lastly, these cloned genes should provide us with new possibilities of developing a convenient, extremely sensitive assay for monitoring clinical populations with regard to the human Ah locus phenotype.

407 CARCINOGENESIS STUDIES USING CULTURED HUMAN EPITHELIAL TISSUES AND CELLS, Curtis C. Harris, Human Tissue Studies Section, National Cancer Institute, Bethesda, MD 20205

Model systems using cultured human epithelial tissues and cells provide several research opportunities including the study of: a) mechanism(s) of carcinogenesis directly in human cells; b) metabolic activation and deactivation of chemical procarcinogens; and c) variations in carcinogen metabolism, carcinogen-DNA adducts and repair of these adducts among different animal species, individuals within a single species, organs and cells, and the relationship, if any, between these differences and susceptibility to the carcinogenic effects of chemicals.

Initial studies have focused on the balance between the activation and the deactivation of chemical procarcinogens and the identification of carcinogen-DNA adducts. Using the formation of adducts as a putative measure of this metabolic balance, representative procarcinogens from diverse chemical classes, i.e., polynuclear aromatic hydrocarbons, N-nitrosamines, mycotoxin and hydrazines, have been investigated in cultured human bronchus, colon, pancreatic duct and esophagus. In general, the metabolic pathways and the carcinogen-DNA adducts are each qualitatively similar among people and also among outbred animal species, however, wide quantitative differences (50- to 150-fold) have been found by us and independently by other investigators. Wide differences in biological response are also found in human tissue- and cell-mediated mammalian mutagenesis assays.

The occurrence of carcinogen-DNA adducts in exposed human populations and the relationship between such adducts and the mechanism(s) of carcinogenesis are two current areas of research interest. The recent development of specific and highly sensitive enzyme immunoassays for the quantitative measurement of carcinogen-DNA adducts in human cells should aid efforts in epidemiology of high cancer risk populations and in the molecular biology of carcinogen-gene interactions.

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Cellular Processing of DNA Damage

408 REPAIR RESPONSES TO DNA DAMAGE: ENZYMATIC PATHWAYS IN E. COLI AND HUMAN CELLS
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Most of our models for DNA repair responses have been derived from the analysis of UV-irradiated bacterial mutants deficient in relevant enzymes. In E. coli the mechanism of excision repair is complex, involving multiple exonuclease and polymerase activities as well as a long patch pathway dependent upon induction of recA protein. Some similarities but notable exceptions are seen when excision repair models derived from E. coli are tested in human cell systems (1). For example, no long patch excision repair is seen in human cells (2). In E. coli the constitutive, short patch excision repair is error free. Long patch repair may be predominantly responsible for the inducible recA-dependent reactivation of double strand DNA genomes (3). Rapid resumption of DNA replication in UV-irradiated bacteria is observed under conditions that permit long patch repair. We hypothesize that long patch repair is a manifestation of damage-processing events at a limited number of unique sites in the genome, in particular, those regions proximal to replication forks and those in regions of active transcription where the DNA duplex structure may be opened up.

In mammalian cells it is likely that the enhanced virus reactivation seen in carcinogen-treated host cells is predominantly due to some response other than excision repair, possibly one that facilitates bypass of persisting damage in the genome (4). This difference between bacteria and mammalian systems may reflect the higher "density" of replication forks per unit DNA in the latter. In eukaryotes there is a much higher probability than in prokaryotes that an excision repair site will overlap a replication fork.

The endonuclease V of bacteriophage T4 can be used as a highly specific and sensitive probe for pyrimidine dimers in DNA (5). Complementation of the excision-repair deficiency in UV-irradiated xeroderma pigmentosum is achieved in permeabilized cells or isolated nuclei upon addition of the enzyme (6). We have cloned the denV gene that codes for the pyrimidine dimer specific glycosylase of T4 endonuclease V (7) and have demonstrated its expression by a number of criteria in uvrA recA and uvrB recA transformants of E. coli.

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409 THE EFFECTS OF DNA DAMAGING AGENTS ON REPLICON INITIATION IN NORMAL AND IN ATAXIA TELANGIECTASIA CELLS,
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The process of initiation of DNA replicons is sensitive to many agents that damage DNA. However, there are at least two kinds of blocks to replicon initiation. One, which is induced by ultraviolet light and aflatoxin B_1 (among others), occurs only at levels of damage that cause at least one lesion per replicon. The second kind, which is caused by X-radiation and bleomycin, occurs at levels of damage where there is only one lesion per cluster of replicons, i.e. one lesion per many replicons. The first kind of agent causes blocks to initiation in both normal diploid cells and in cells from patients with ataxia telangiectasia; the second kind of agent strongly blocks initiation of replicons in normal cells but only very weakly in ataxia telangiectasia cells. Studies with ataxia telangiectasia cells show that both the cell cycle delay caused by blocks to replicon initiation and the cell cycle delay caused by blocks from G_2 into mitosis are under genetic control. Work supported by the U.S. Department of Energy.

410 THE PARTICIPATION OF (ADP-RIBOSE) IN DNA REPAIR, Sydney Shall, Barbara Durkacz, Mohan Perera, Biochemistry Laboratory, University of Sussex, Brighton, Sussex, U.K.

DNA-damaging chemicals and radiation lower cellular NAD levels. We provide a biochemical explanation for these observations and we show that DNA excision repair in eukaryotes requires NAD for the synthesis of $(ADP\text{-}ribose)_n$. The biosynthesis of $(ADP\text{-}ribose)_n$ is absolutely dependent on the presence of DNA; the ability of DNA to activate the enzyme is proportional to the number of nicks in the DNA. Fragmentation of the DNA increases its ability to activate the enzyme.

The decrease in cellular NAD caused by radiation or chemicals (e.g. DMS, MNU, MMS, EMS, BCNU, MeCCNU, BMBA, streptozotocin, neocarcinostatin) can be prevented by all four classes of inhibitors (e.g. 3-amino benzamide, 5-methylnicotinamide, theophylline, theobromine, IBMX, or thymidine) of $(ADP\text{-}ribose)_n$ biosynthesis. The specific activity of $(ADP\text{-}ribose)_n$ polymerase is increased by all the above DNA-damaging agents in a dose-dependent way. Moreover, the kinetics of NAD change and enzyme activation correpond. We conclude that DNA-damaging agents cause the utilisation of NAD for the synthesis of chromatin-bound $(ADP\text{-}ribose)_n$ (1-3).

The inhibitors of $(ADP\text{-}ribose)_n$ biosynthesis retard the rejoining of single-strand breaks in DNA as detected by alkaline sucrose gradients, alkaline elution or the nucleoid technique. Partial depletion of cellular NAD by nicotinamide starvation, totally prohibits rejoining of single-strand breaks. Both the enzyme inhibitors and NAD depletion synergistically potentiate the cytotoxicity of DNA-damaging agents. One of these enzyme inhibitors, 3-amino benzamide, seems to be the first specific inhibitor of DNA repair (4,5).

It seems that the site of involvement of $(ADP\text{-}ribose)_n$ in DNA excision repair is one of the later steps. Our evidence shows that $(ADP\text{-}ribose)_n$ is involved after the incision step. The participation of $(ADP\text{-}ribose)_n$ in DNA excision repair is demonstrated in both rodent and human cells, in both normal and tumour cells and in a variety of cell types. Furthermore, it has already been reported that one of the human genetic diseases, Ataxia telengiectasia, defective in DNA repair is also defective in $(ADP\text{-}ribose)_n$ response to DNA damage.

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411 DNA Replication in UV-Irradiated Human Fibroblasts with Hypersensitivity to DNA Damage including Xeroderma Pigmentosum (XP) and Cockayne's Syndrome. Cleaver, J.E. and Kaufmann, W.K., Laboratory of Radiobiology, University of California, San Francisco, California 94143

DNA replication and repair of DNA damage are coupled processes that may augment or diminish mutagenesis, sister chromatid exchanges (SCEs), and carcinogenesis in damaged human cells. Several inherited disorders, e.g. XP, XP variant, Cockayne's syndrome (CS), and ataxia telangiectasia (AT), are hypersensitive to DNA damage and some of these have alterations in replication or repair. UV-induced damage and repair rapidly change both the number of replications that can initiate DNA synthesis and the progression of replication forks in normal cells. At low doses (< 1 J/m^2) reduction in initiation predominates: higher doses (> 3 J/m^2) are needed to impede fork progression. Repair deficient XP cells show less inhibition of replicon initiation and greater inhibition of fork progression. XP variant cells show a greater inhibition of initiation at low doses and greater inhibition of fork progression at higher doses. Only for the XP variant does the length of nascent DNA strands correspond to the spacing of lesions in DNA, suggesting that these cells have lost a gene product that enables other cell types to replicate without interruption past certain damaged sites. All excision defective XP groups show less recovery of DNA replication than normal, but although both groups C and D have 10-20% repair, DNA replication recovers much faster in group C than in group D indicating a qualitative difference in this repair. DNA replication fails to recover in UV-irradiated Cockayne's syndrome and XP variant cells, but for different reasons even though both exhibit normal excision repair. Bloom's syndrome, which exhibits high spontaneous carcinogenesis, SCEs and mutagenesis, shows no major abnormalities in repair or replication. These varied responses indicate that inhibition and recovery of DNA replication as well as repair of damaged DNA are under genetic control. Since both the excision defective and variant forms of XP show high levels of UV induced skin cancer and of point mutations *in vitro*, but only excision defective cells show high levels of sister chromatid exchange, carcinogenesis appears to correlate with mutagenesis rather than gene rearrangement. The apparent paucity of internal cancers in XP may merely indicate that internal exposure to chemical carcinogens is relatively small compared to the intense exposure to sunlight on the skin. Work supported by the U.S. Department of Energy.

412 ERROR-FREE EXCISION REPAIR BY HUMAN CELLS OF THE POTENTIALLY CYTOTOXIC AND MUTAGENIC DNA ADDUCTS FORMED BY CARCINOGENS, Veronica M. Maher and J. Justin McCormick, Carcinogenesis Laboratory, Fee Hall, Michigan State University, East Lansing, MI 48824.

The ability of normal diploid human fibroblasts and excision repair deficient xeroderma pigmentosum cells to excise potentially cytotoxic or mutagenic lesions induced in DNA by a series of carcinogenic agents was investigated. Large populations of cells were prevented from replicating by being grown to confluence. After 3 days without refeeding the cells were irradiated or exposed for 2 hr to radioactive labeled compounds. One set of cultures was immediately released and assayed for the number and kind of residues covalently bound to DNA; for percent survival; and/or for the frequency of induced mutations. After various periods of time in confluence, other sets were similarly released and assayed. The normal cells exhibited a gradual loss of DNA adducts from their DNA, a gradual increase in survival, and a gradual decrease in the induced mutation frequency. For the majority of agents tested, the excision repair deficient cells showed no loss of adducts and no change in survival or mutation frequency with time held in confluence. We are using this technique to identify the specific DNA adduct(s) responsible for the cytototoxic and/or mutagenic effects of a series of carcinogens, including reactive derivatives of polycyclic aromatic hydrocarbons and aromatic amides, simple alkylating agents, UV radiation, etc. The data strongly suggest that the cytotoxic and mutagenic lesion formed by the anti isomer of the 7,8-diol-9,10-epoxide of benzo(a)pyrene is the N^2 guanine adduct; that of N-acetoxy-2-acetylaminofluorene is the deacetylated C-8 guanine adduct; and that of UV radiation is the pyrimidine dimer. (Supported by NIH Grants CA 21247 and CA 21253.)

413 CHROMOSOME DAMAGE, DNA REPAIR, AND SINGLE-GENE MUTATION, Anthony V. Carrano and L.H. Thompson, Biomedical Sciences Division, Lawrence Livermore National Laboratory, Livermore, CA 94550

The biological response of a cell to insult is governed by at least two factors: the nature of the initial lesions and the manner in which the cell converts the lesions to biologically significant events. Our approaches to understand these events have been to: 1) compare quantitatively the efficiency of a variety of lesions for inducing both sister chromatid exchange (SCE) and single-gene mutation in Chinese hamster ovary (CHO) cells; 2) identify the nature of the lesion and role of chromatin type in eliciting the SCE; and 3) examine the induction and persistence of SCE in CHO repair-deficient cells. We have investigated the dose response characteristics for nine chemicals consisting of mono-and bi-functional alkylating and intercalating agents. In addition, we used two physical agents, UV light and ionizing radiation. For seven of the chemicals and for the two radiation types, the induction of both SCE and mutation at the hprt locus in CHO cells was linear. When the frequency of induced SCE was plotted against induced mutation, the relation was linear. The slopes, however, were unique for each agent indicating that the induction of both endpoints is lesion dependent. Further evidence to support this comes from studies with repair-deficient CHO cells which are hypersensitive to killing by UV light and chemical mutagens such as 7-bromomethylbenz(a)anthracene (BMBA). These cells demonstrated an increased sensitivity per unit dose to the induction of both SCE and mutation (hprt and aprt loci). In addition, the lesions which lead to the formation of UV-induced SCEs appear to persist longer in repair-deficient cells than in normal CHO. For BMBA, the frequency of both SCE and mutation, as a function of surviving fraction, is greater in the repair-proficient cells than in the mutant strains. This suggests that the ultimate genetic effects may reflect different efficiencies of repair of potentially mutagenic lesions vs potentially lethal lesions.

Specific attention has been given to the induction of SCEs by mitomycin-C (MMC) in the euchromatin and heterochromatin of the Indian muntjac. Both baseline and MMC-induced SCEs are suppressed in hetero- vs euchromatin. Initial results suggest that the rate of removal of the SCE-forming lesions are similar in the two chromatin types. Preliminary studies in human fibroblasts extend our earlier findings that the DNA-DNA crosslink may not be the lesion responsible for the formation of SCEs in vitro by MMC. (This work was supported by U.S. DOE Contract No. W-7405-ENG-48 at Lawrence Livermore National Laboratory.)

In Vivo and In Vitro Carcinogenesis

414 INITIATION AND PROMOTION OF RADIATION TRANSFORMATION IN MOUSE FIBROBLASTS, John B. Little, Harvard School of Public Health, Boston, MA 02115

Mouse cell lines can be transformed in a dose dependent manner by exposure to x-rays, alpha particles, or auger electrons from ^{125}I incorporated into DNA as $^{125}IUdR$ (1). When BALB/3T3 cells were irradiated in stationary growth and subculture to low density delayed, the yield of transformants declined rapidly in X-irradiated cells but showed no change with recovery intervals up to 120 hrs following alpha irradiation. These results suggest that DNA damage is important in the initiation of transformation by radiation.

Transformation can be enhanced by post-irradiation incubation with the tumor promoter TPA (2). C3H 10T½ cells were seeded at low density, irradiated, and incubated with or without TPA during the expression period. TPA was effective primarily during the proliferative phase of expression (first 10-14 days). However, a similar enhancement in transformation occurred if the irradiated cells were subcultured and reseeded at low density when they reached confluence, and TPA added 12-13 cell generations after x-ray exposure. These results suggest the following conclusions concerning the promoting effect of TPA in established mouse cell lines: 1) it is not due to a conversion of premutational DNA lesions to mutations or to an effect on DNA repair processes; 2) it is not due to a simple stimulation of cell proliferation, though TPA is effective primarily in proliferating cells; and 3) it does not result primarily from stimulating quiescent cells to proliferate nor releasing cells from contact inhibition.

In other experiments (3), cultures were irradiated after initially seeding them at varying cell densities (1 to 300 viable cells), or they were seeded and irradiated at low density, harvested when they reached confluence and reseeded at 10-fold dilutions down to 1:10,000. The unexpected finding was that the average number of transformed foci which developed per confluent dish was roughly constant over a wide range of initial and reseeded cell densities; that is, it was independent of the number of cells exposed to the radiation or the number of progeny of irradiated cells reseeded after confluence. These results suggest that the initial alteration produced by radiation is a common event involving most or all of the irradiated cells. We hypothesize that it may be epigenetic in nature, such as an alteration in gene expression. This initial alteration enhances the probability that the actual transformation of one or more of the progeny of the irradiated cells will occur later on when they are maintained under conditions of confluence.

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415 TUMOR PROMOTION AND PROMOTER INDUCED CELLULAR CHANGES IN MOUSE EPIDERMAL CELLS. Stuart H. Yuspa, Ulrike Lichti, Molly Kulesz-Martin and Henry Hennings, Laboratory of Experimental Pathology, National Cancer Institute, Bethesda, Maryland 20205. Mouse epidermal basal cells can be selectively cultivated in medium with a calcium concentration of 0.02 - 0.09 mM. Terminal differentiation and sloughing of mature keratinocytes occurs when the calcium concentration is increased to 1.4 mM. This model system has provided a means to determine if basal cells are the primary target for phorbol esters in epidermis and to explore the possibility that heterogeneity of response exists within subpopulations of basal cells. The induction of the enzyme ornithine decarboxylase (ODC) was utilized as a marker for responsiveness to phorbol esters. ODC induction after exposure to 12-O-tetradecanoylphorbol-13-acetate (TPA) in basal cells is enhanced 20 - 40 fold over the response of a culture population containing both differentiating and basal cells. When basal cells are induced to differentiate by increased calcium, responsiveness to TPA is lost within 6 hours. In basal cell cultures, two ODC responses can be distinguished. After exposure to low concentrations of TPA or to weak promoters of the phorbol ester series, ODC activity is maximal at 3 hours. With higher concentrations of TPA, the ODC maximum is at 9 hours. These results are consistent with the presence of subpopulations of basal cells with differing sensitivities to TPA. Separate studies which utilize the enzyme epidermal transglutaminase as a marker for differentiation support this conclusion. In basal cell culture TPA exposure rapidly increases transglutaminase activity reflecting induced differentiation in some cells. As differentiated cells are sloughed from the dish, residual basal cells proliferate and become resistant to induced differentiation by 1.4 mM calcium. These data provide additional evidence of basal cell heterogeneity in which one subpopulation is induced to differentiate while another is inhibited. When basal cell cultures are exposed to chemical initiators of carcinogenesis, colonies of cells which resist calcium induced differentiation evolve. Likewise basal cells derived from mouse skin initiated in vivo yield foci which resist terminal differentiation. This defect in the commitment to terminal differentiation appears

to be an essential change in initiated cells in skin and is also characteristic of malignant epidermal cells. Tumor promoters may act to redistribute subpopulations of epidermal cells due to heterogeneous responses with regard to terminal differentiation. Cells resistant to terminal differentiation, such as initiated cells, would be expected to increase in number during repopulation. Clonal expansion of the initiated population could result in a benign tumor whose major characteristic is an altered program of differentiation.

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416 SEQUENTIAL BIOLOGIC AND BIOCHEMICAL ALTERATIONS DURING HEPATOCARCINOGENESIS, Frederick F. Becker and Daniel L. Stout, Department of Pathology, University of Texas System Cancer Center, Houston, TX, 77024

Although the concept that initiation and promotion are the major components of carcinogenic evolution may be valid, their nature remains ill-defined. However, by manipulation of carcinogenic regimens one can seek to identify the obligate factors in this progression. We have identified a putative, premalignant lesion, the persistant hepatic nodule, which is induced by a high dose, discontinuous exposure to the aromatic amide, N-2-acetylaminofluorene (1). Utilizing a modification of the alkaline elution technique, it was demonstrated that a significant fraction of the nuclear DNA from cells of these nodules eluted more rapidly than that of controls (2). This early eluting fraction, which has been proposed to represent "short", single-stranded DNA, increased with time despite cessation of the carcinogen.

To determine whether this phenomenon was general in nature we applied alkaline elution to livers which had received a single dose of 6.55 mg/k DEN during the regenerative response to partial hepatectomy, and subsequent, chronic exposure to phenobarbital (regimen of Pitot). After 7 months of phenobarbital the elution pattern of nuclear DNA became progressively abnormal, demonstrating a significant fraction of early eluting material. Concurrently, foci of hepatocytes appeared which were histochemically altered in a pattern suggested to represent a phase of malignant progression. The possible role of DNA alteration in carcinogenic progression will be discussed.

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417 DYNAMICS OF NEOPLASTIC DEVELOPMENT IN TRACHEAL EPITHELIAL CELLS EXPOSED TO CARCINOGENS, Paul Nettesheim, NIEHS, Research Triangle Park, NC, 27709 and Margaret Terzaghi, ORNL, Oak Ridge, TN, 37830

Qualitative and quantitative cellular changes occurring in tracheal epithelium *in vivo* during the process of carcinogenesis were analyzed by using an *in vitro* assay: the epithelial focus assay (EF-assay). Rat tracheas were exposed *in vivo* to either a carcinogenic polycyclic hydrocarbon or to a systemic nitrosamine which causes carcinomas in tracheas of rats receiving the carcinogen in drinking water. Epithelial cells were collected from tracheas at various times after carcinogen exposure and seeded into culture dishes. The culture conditions were such that normal epithelial cells would not survive for more than one month. However, in a high proportion of plates seeded with epithelial cells from carcinogen-exposed tracheas, proliferating epithelial foci (EF) developed. As a function of time after carcinogen exposure, the proportion of EF which could be subcultured (EF_S) and that became anchorage independent and neoplastic (EF_n) increased. So did the frequency of tracheas containing EF_n populations. These studies show that alteration of growth control is an early marker of initiated cells which can be exploited for purposes of selecting different types of "transformed" cells. They also show that *in vivo* only a fraction of the cells endowed with neoplastic potential gives rise to neoplasms. These and other studies concerned with the progression and promotion of neoplastic development *in vivo* and *in vitro* will be discussed.

418 STUDIES ON THE MECHANISMS INVOLVED IN MULTISTAGE CARCINOGENESIS IN MOUSE SKIN.* T.J. Slaga, S. M. Fischer, C. E. Weeks and A.J.P. Klein-Szanto, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830

Skin tumors in mice can be induced by the sequential application of a subthreshold dose of a carcinogen (initiation phase) followed by repetitive treatment with a noncarcinogenic tumor promoter. The initiation phase requires only a single application of either a direct or indirect carcinogen and is essentially an irreversible step which probably involves a somatic cell mutation as evidenced by a good correlation between the carcinogenicity of many chemical carcinogens and their mutagenic activities. There is a good correlation between the skin tumor initiating activities of several polycyclic aromatic hydrocarbons (PAH) and their ability to bind covalently to epidermal DNA. Results from our laboratory as well as others suggest that "bay region" diol epoxides are the ultimate carcinogenic form of PAH carcinogens. Potent inhibitors and stimulators of PAH tumor initiation appear to effect the level of the PAH diol epoxide bound to specific DNA adducts. The phorbol ester tumor promoters have been shown to have several cellular and biochemical effects on the skin. Of all the observed phorbol ester related effects on the skin, the induction of epidermal cell proliferation, polyamines, prostaglandins and dark basal keratinocytes as well as other embryonic conditions appear to correlate the best. Mezerein, a weak promoter, was found to induce many cellular and biochemical changes similar to TPA especially epidermal hyperplasia and polyamines, however, it was not a potent inducer of dark cells. We recently found that promotion could be divided into at least two stages. The first stage (I) is brought about by limited treatment of TPA and the second stage (II) by repetitive applications of mezerein. Fluocinolone acetonide (FA) was found to be a potent inhibitor of stage I and II. Retinoic acid (RA) was ineffective in stage I but was a potent inhibitor of stage II promotion; whereas tosyl phenylalanine chloromethyl ketone (TPCK) specifically inhibited stage I. In addition, FA and TPCK effectively counteracted the appearance of dark basal keratinocytes but very little effect on polyamines, whereas RA had no effect on dark cells but is a potent inhibitor of TPA-induced ornithine decarboxylase activity and subsequent putrescine formation. These results provide additional evidence for the importance of dark basal keratinocytes (primitive stem cells) in stage I of promotion and indicate that most of the other cellular and biochemical responses normally associated with promotion (such as polyamines) are actually associated with stage II of promotion.

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419 EMBRYOLOGIC CONTROL OF MALIGNANCY, G. Barry Pierce, Cooley G. Pantazis, James E. Caldwell, Robert S. Wells, Department of Pathology, University of Colorado School of Medicine, Denver, Colorado 80262

Our operative concept is that carcinoma is a caricature of the normal process of tissue renewal. The target of carcinogenesis is the normal stem cell, which is converted to a malignant stem cell. Some of the offspring of malignant stem cells have the capacity to differentiate into benign, if not normal, cells and tissues. This effect is best seen in testicular teratocarcinomas. The malignant stem cells of this tumor, embryonal carcinoma cells, resemble and have histochemical reaction in common with inner cell mass cells of the blastocyst, and like the inner cell mass cells are capable of differentiation. This propensity for differentiation raises the possibility that direction of differentiation might serve as an alternative to the usual type of cytotoxic therapy for the individual with carcinoma with metastasis.

This idea has received support from the experiments of Brinster, Mintz, Illmensee, and Papaioannou, who showed that if an embryonal carcinoma cell is injected into the blastocyst of a mouse and the injected blastocyst is placed in the uterus of a pseudopregnant female carrier, a chimeric mouse is born. Thus, the blastocyst is capable of regulating an embryonal carcinoma cell making it behave as a normal embryonic cell. We have developed an assay based upon the comparison of tumorigenicity of embryonal carcinoma cells injected into animals within blastocysts or alone. To date, the assay indicates that the blastocyst can control a single embryonal carcinoma cell provided it is placed within the blastocoele cavity; however, it is incapable of regulating 3, 4, or 5 of them. The reaction is specific in that the blastocyst cannot regulate melanoma cells, leukemia cells, or sarcoma cells, but it can regulate neuroblastoma cells. Since neurulation follows blastulation by 96 hours, it is possible that the neuroblastoma cells are controlled at the time of neurulation. Currently, the blastocyst is being dissected to determine which of its components is responsible for the regulation.

It is our postulate that if one embryonic field can regulate its closely corresponding type of carcinoma, there will be an embryonic field for each type of cancer.

Genetics of Malignancy

420 REGULATION OF THE EXPRESSION OF VIRAL TUMOR ANTIGEN IN TERATOCARCINOMA STEM VERSUS DIFFERENTIATED CELLS, Carlo M. Croce, Alban Linnenbach and Kay Huebner, The Wistar Institute, 36th Street at Spruce, Philadelphia, Pa. 19104.

In order to study the molecular basis of regulation of gene expression in stem versus differentiated cells we have produced recombinant thymidine kinase vectors carrying either SV40 or Polyoma virus DNA to transform thymidine kinase deficient mouse teratocarcinoma stem cells. Stem cell transformants that contained the recombinant DNA vectors integrated in the chromosomal DNA did not express viral tumor antigens (1-2). Following their induction to differentiate with retinoic acid, however, all the transformants expressed these antigens (1-2). We are presently investigating the basis for the differential gene expression in stem versus differentiated cells.

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421 SOME EVIDENCES FOR RELATIONSHIP BETWEEN INDUCTION OF MUTATION AND NEOPLASTIC TRANSFORMATION BY CHEMICAL CARCINOGENS IN MAMMALIAN CELLS.
Takeo Kakunaga, Hiroshi Hamada, John Leavitt*, Ko-Yu Lo and Tadashi Hirakawa, Cell Genetics Section, Laboratory of Molecular Carcinogenesis, NCI, NIH, Bethesda, MD 20205 and *Division of Virology, Food and Drug Administration, Bethesda, MD 20205

There have been many circumstantial evidences to support the close relationship between carcinogenesis and mutagenesis or teratogenesis. However, no cellular molecules have been unequivocally identified as factors directly involved in the induction of cell transformation. In order to identify these factors, two approaches have been attempted; 1) isolation and characterization of cell mutants which are affected in the initiation or expression of cell transformation, and 2) identification of cellular genes and their products controlling the normal or transformed phenotypes.

Ts- and Cs-mutants for anchorage-independent growth in gel were isolated from a Chinese hamster lung cell line(1). Cell variants showing different susceptibility to UV-transformation were isolated from a subclone of Balb/3T3 line(2). The different susceptibility of the Balb/3T3 variants to transformation was also observed when various chemical carcinogens were used as the transforming agents. The results on the susceptibility of the variants to the killing effects of carcinogens, the transformation by RNA tumor virus, the induction of ouabain-resistant mutation by carcinogens, formation and removal of carcinogen-DNA adduct, confluent-holding of carcinogen-treated cells, and effects of promoting agents suggest that fixation of transformation occurs at similar frequencies between variants via DNA damage, and that expression step following fixation step is a major step for determining the susceptibility of variant cells to transformation by chemical carcinogens and UV.

When chemically transformed human fibroblasts and the untransformed parental cells were compared by two-dimensional gel electrophoresis of their protein, one new polypeptide was recognized in the proteins from a transformed line(3). This new polypeptide was identified as a product of mutated β-actin gene by immunoprecipitation with anti-actin antibody, comparison of its tryptic peptide patterns, isolation of mRNA which codes for the new protein, cloning of cDNA complementary to actin mRNA from the transformed cells into plasmid, Southern blotting analysis of actin gene of transformed cells using newly cloned plasmid which contains human β-actin cDNA and a recombinant plasmid DNA which contains dictyostelim actin cDNA as probe. These results seem to provide the first evidence that at least one of the protein changes observed in the transformed cells is due to the mutation.

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422 TERATOCARCINOMA MODELS FOR STUDYING DIFFERENTIATION AND NEOPLASIA, John M. Lehman, Thomas Friedrich and Katrina Trevor, Department of Pathology, University of Colorado Health Sciences Center, Denver, CO 80262.

The murine teratocarcinoma is a complex tumor composed of the malignant stem cell, embryonal carcinoma (EC), and differentiated cells representative of all three embryonic germ layers. Further, work has demonstrated that the EC cell is multipotential, able to differentiate into these multiple cell types, and capable of contributing to the development of a mouse. This tumor has been adapted to tissue culture where it exhibits similar patterns of growth and differentiation as observed in the solid tumors (1). Therefore, this system offers potential for studies concerning differentiation, gene regulation and neoplasia. Our studies have focused on the response of this cell system to infection with the two papovaviruses, Simian virus 40 and polyoma (2). The differentiated cells respond by supporting replication of polyoma virus and transformation with SV_{40} virus; however, the EC cells show no evidence of infection. Numerous studies have demonstrated that the block to infection of the EC cell is not at adsorption, penetration, uncoating or related to interferon production (3,4). In fact, the viral DNA can be demonstrated in the EC cells in an unintegrated state and attempts to detect viral DNA replication have been negative, suggesting that the viral genome is lost with subsequent cell replication. We next characterized the fate of the SV_{40} genome in infected EC cells that were either spontaneously or chemically induced to differentiate. The experiments demonstrated that these differentiated cells did not express viral proteins. However, the viral DNA was present in the differentiated cells as demonstrated by Southern gel analysis and transfection of this DNA onto permissive CV-1 cells. Thus, the viral DNA in EC cells is in some way modified, preventing expression of the viral DNA. These studies and others will be discussed to better define the mechanism of this block to virus infection in the murine teratocarcinoma cell.

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423 CARCINOGEN MEDIATED AMPLIFICATION OF SPECIFIC DNA SEQUENCES IN CHINESE HAMSTER CELLS, Sara Lavi, The Weizmann Institute of Science, Rehovot, Israel.

Studies on the effect of carcinogens on amplification of genomic sequences led us to the design of a model experimental system based on Chinese hamster cells transformed by SV40 which contains exclusively integrated SV40 DNA sequences. Treatment of these cells with a variety of chemical and physical carcinogens induced SV40 DNA synthesis in very high proportion of the cells. When Chinese hamster cells transformed by temperature sensitive mutant of SV40 (tsA209) were treated with carcinogens,induction occurred only at the permissive temperature indicating that the amplification of SV40 sequences is controlled by the viral A gene and is not a reflection of nonspecific DNA repair.

Analysis of the amplified DNA by Southern's hybridization revealed that the SV40 specific sequences are heterogenous in size ranging from molecules which are several folds larger than the intact SV40 genome to very short DNA fragments. Restriction endonuclease digestion of the induced DNA molecules indicated that the host sequences adjacent to the SV40 sequences were also amplified.

DNA extracted from the carcinogen treated cells was used for transformation of secondary Chinese hamster embryo cells. The transformation frequency with DNA isolated from carcinogen treated cells was several folds higher than that by DNA extracted from untreated control cells. The possible role of the DNA molecules amplified by carcinogens in cell transformation will be discussed.

Molecular Biology of the Genetic Apparatus

424 MOLECULAR BIOLOGICAL STUDIES ON DNA REPLICATION: CURRENT STATUS AND FUTURE PROSPECTS, Bruce M. Alberts, Jack Barry, Patricia Bedinger, Rae Lyn Burke and Kenneth N. Kreuzer, Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143

Several different multienzyme systems capable of efficient *in vitro* DNA replication have recently been reconstructed from purified components, in work involving a number of different laboratories. Reactions catalyzed by the separate sets of replication proteins encoded by bacteriophage T7, by bacteriophage T4 and by the *E. coli* host have thus far been the most extensively characterized (reviewed in Kornberg, A., *DNA Replication*, W.H. Freeman & Co., San Francisco, 1980). The results obtained in these three prokaryotic *in vitro* systems have important implications for future studies on eukaryotic cells.

Our own work has been carried out with T4 bacteriophage DNA replication proteins. The T4 *in vitro* replication system involved in replication fork movement, as presently constituted, consists of 7 highly purified T4-induced proteins. The protein components can be grouped into functional classes as follows: DNA polymerase (gene 43 protein), helix-destabilizing protein (gene 32 protein), polymerase accessory proteins (gene 44/62 & 45 proteins), and RNA priming proteins (gene 41 & 61 proteins). Mutants in each of the corresponding genes have been shown by others to have a severe effect on the *in vivo* replication process. DNA synthesis in this 7 protein *in vitro* system starts by covalent addition onto the 3'OH end at a nick on a double-stranded DNA template, and proceeds to generate a replication fork which moves at about the *in vivo* rate, and with approximately the *in vivo* base-pairing fidelity. DNA is synthesized at the fork in a continuous fashion on the leading strand and in a discontinuous fashion on the lagging strand (generating short Okazaki fragments with 5'-linked pppApCpXpYpZ penta-ribonucleotide primers).

Both the 44/62 protein complex and the 41 protein catalyze a single-stranded DNA-dependent hydrolysis of nucleoside triphosphates to nucleoside diphosphates and inorganic phosphate; these hydrolyses can be differentially blocked by ATPγS and by GTPγS respectively, revealing that each hydrolysis has an essential role in a functioning replication fork. The DNA synthesis on the leading and lagging side of the fork are linked via the dual role of the gene 41 protein: this protein appears to use nucleotide hydrolysis energy to run along the lagging-strand template at the fork, simultaneously helping to drive open the parental DNA helix (to permit rapid leading-strand synthesis), while acting as a mobile site at which new RNA primers are formed (to prime Okazaki piece synthesis on the lagging strand). In a second reaction requiring nucleotide hydrolysis, the gene 44/62 and 45 proteins act in a synergistic manner to tie down the DNA polymerase, causing it to move through these double-helical templates in an enormously processive fashion.

Growth Factors for Normal, Preneoplastic and Malignant Cells

425 MOLECULAR BASIS FOR THE ORIGIN OF HIGH AFFINITY EGF BINDING IN CULTURED CELLS: EFFECT OF TPA ON THE GENERATION OF HIGH AFFINITY EGF RECEPTORS IN BALB/c 3T3 CELLS AND ISOLATED MEMBRANES. C. Fred Fox and Kenneth K. Iwata, Department of Microbiology and Molecular Biology Institute, University of California, Los Angeles, CA 90024

There are two EGF binding components on the surfaces of numerous human and mouse clonal cell lines and cell strains. High and low EGF binding affinities also were observed in cell surface membrane preparations derived from human or murine cells showing that the existence of both binding affinities does not require actively metabolizing cells and cannot be explained by EGF and receptor internalization. We undertook a study to determine the molecular basis for generation of high affinity EGF binding. Conditions have been established in both cells and membranes which limit EGF binding to low affinity binding sites only. Different conditions, e.g., exposure of cells to unlabeled EGF prior to ^{125}I-EGF binding favor the formation of high affinity binding sites which appear to be generated from existing low affinity sites. The generation of high affinity EGF binding is temperature and time dependent and requires an energy source. It has been demonstrated in cells and in an in vitro system with isolated membranes. Generation of high affinity EGF binding in membranes requires purine nucleosoide triphosphates.

There have been several reports that cells exposed to active tumor promoting agents such as TPA (12-O-tetradecanoyl-phorbol-13-acetate) lack high affinity EGF binding. We extended our studies to explore the mechanism by which TPA affects high affinity EGF binding. TPA inhibited the formation of high affinity EGF binding in cells; its influence on the generation of high affinity EGF binding in membranes is currently under study. The possible relationship between these findings and the role of TPA as a tumor promoter will be discussed.

426 CALCIUM-CALMODULIN REGULATION IN NORMAL AND MALIGNANT CELLS, A.R. Means, J.G. Chafouleas, B.R. Brinkley, R.L. Pardue, W.E. Bolton, A.E. Boyd, III, Depts. of Cell Biology and Medicine, Baylor College of Medicine, Houston, TX 77030.

Calmodulin (CaM) regulates cyclic nucleotide and glycogen metabolism, protein phosphorylation, Ca^{2+} flux, cell motility and division (1). CaM also affects the state of microtubule (MT) polymerization by promoting disassembly in a Ca^{2+} dependent manner (2). Many malignant cells exhibit a diminished cytoplasmic microtubule network (CMTC) compared to their non-transformed counterparts. We have determined by a specific radioimmunoassay that CaM is elevated 2-3 fold in many malignant cells. Studies in 2 cell lines, 3T3-SV3T3 and NRK-SNRK, have shown that the increase occurs at the level of synthesis and not degradation (3). Tubulin content is not different between normal and transformed cells. Thus the diminished CMTC could be due to the 2 x increase in CaM to tubulin ratio. A detergent-treated cell system was established to study the assembly of MT from the cytoplasmic organizing center (4). SV3T3 cells were attenuated in their ability to nucleate MT assembly compared to 3T3 cells. This difference could be overcome by preincubation of the SV3T3 cell with anti-CaM before tubulin addition. These data argue that CaM-mediated regulation may be directly related to its effective concentration in the cell at any given time. Studies were undertaken to evaluate fluctuations in CaM during the cell cycle. CHO-K1 cells were synchronized by mitotic shake and CaM levels were determined by RIA during the subsequent cell cycle. CaM levels at mitosis are 150 ng/10^6 cells and fall by 50% in early G_1. During late G_1 CaM increases to the M value and remains at this concentration for the duration of the cell cycle. Subsequent experiments with cell populations that varied in the length of G_1 revealed that CaM always doubled in late G_1 or early S suggesting that CaM might be important in the G_1/S transition. This possibility was investigated by using the anti-CaM drug W13 and its inactive homolog W12 (5). W13 resulted in a 72% reduction in cell number after 24 hr and flow cytometry revealed a build-up of cells in G_1 at the expense of the S population. The drug caused no cytoxicity and the cell cycle block was readily reversible. These data argue that reduction in the effective CaM level results in a decreased ability of cell progression into S and predict an important regulatory role for CaM in the G_1/S transition.

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427 TRANSFORMING GROWTH FACTORS (TGFs) PRODUCED BY CERTAIN HUMAN TUMOR CELLS: POLYPEPTIDES THAT INTERACT WITH EPIDERMAL GROWTH FACTOR (EGF) RECEPTORS, George J. Todaro, Charlotte Fryling and Joseph E. De Larco, National Cancer Institute, National Institutes of Health, Frederick, MD 21701

Three different human tumor lines in culture, a rhabdomyosarcoma, a bronchogenic carcinoma and a metastatic melanoma, release proteins (transforming growth factors, TGFs) into the medium that confer the transformed phenotype on untransformed fibroblasts. These proteins are acid and heat stable, produce profound morphologic changes in rat and human fibroblasts and enable normal anchorage-dependent cells to grow in agar. Removal of the transforming protein results in a reversion of cell phenotype. The major activity interacts with epidermal growth factor (EGF) cell membrane receptors. The peptides from these tumor cells are similar in their action to sarcoma growth factor (SGF) released by murine sarcoma virus-transformed rodent cells. The most anchorage-independent tumor cells also released the most TGFs. EGF-related TGFs were not detectable in fluids from cultures of cells with high numbers of free EGF membrane receptors (normal human fibroblasts and human carcinomas).

428 PRENEOPLASTIC CELL PHENOTYPE: RESPONSES TO TUMOR PROMOTERS AND GROWTH FACTORS, Nancy H. Colburn, L. David Dion, Leela Srinivas, Edmund J. Wendel, Laboratory of Viral Carcinogenesis, NCI, Frederick Cancer Research Center, Frederick, MD 21701

The "promotable" mouse epidermal cell line JB6 is being used as a model for studying the mechanism of late stage tumor promotion. JB6 cells respond to tumor promoting phorbol diesters by undergoing a progression to tumor cell phenotype as measured by anchorage independence and tumorigenicity (1). The process is irreversible (1) and occurs by a mechanism involving induction of new phenotype(s) (2). Promotion of anchorage independence occurs in response to several classes of tumor promoters including phorbol diesters, mezerein, cigarette smoke and detergents (3) and to epidermal growth factor (EGF) (3) and transforming growth factors of DeLarco and Todaro, but not in response to the calcium ionophore A23187, a first stage promoter (Slaga et al.). We have obtained by colchicine selection against mitogen sensitive cells a set of variants derived from a JB6 clone which are resistant to mitogenic stimulation by TPA at plateau density. Three of these mitogen resistant variants are sensitive to promotion (M-P+), thus suggesting that this type of promoter-dependent mitogenic stimulation can be ruled out as a requirement for late-stage promotion. To date neither the M-P+ nor independently isolated mitogen sensitive nonpromotable (M+P-) variants showed a deficiency in levels of phorbol diester receptors. We are currently determining whether differences in receptor affinity or down modulation can account for differences in promoter responsiveness. That the nonpromotability of the M+P- variants may reflect a phorbol ester receptor-independent defect is suggested by the observation that the variants which are resistant to promotion by TPA are also resistant to promotion by cigarette smoke and EGF (3). Variants which were deficient in either the mitogenesis or the promotion response to TPA showed reduced levels of EGF binding in the absence of TPA and reduced recovery of EGF binding after down modulation by TPA. Two major changes in glycoconjugate synthesis occur in JB6 cell lines in response to tumor promoting but not non-promoting phorbol esters. The first is a decrease in incorporation of tritiated mannose or proline into procollagen which occurs in response to promoting concentrations of TPA and is antagonized by antipromoting concentrations of retinoic acid. The level of TPA action appears to be transcriptional. Promotion sensitive cells show significantly lower basal levels of procollagen than promotion resistant variants. A second promoter induced change in glycoconjugate synthesis is a 10-fold reduction in ^{14}C-glucosamine incorporation into trisialogangliosides, the major gangliosides found in JB6 cells. A model is proposed in which changes in membrane gangliosides and collagen secretion play roles in signal transmission following phorbol ester receptor binding.

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429 CELLULAR INTERACTIONS AND THE EXPRESSION OF THE MALIGNANT PHENOTYPE. J.S. Bertram, B.B. Bertram and P. Janik, Cancer Drug Center, Roswell Park Memorial Institute, Buffalo, NY 14263

Neoplastic transformation of 10T½ cells is characterized by a loss of contact inhibition of growth, loss of anchorage dependent growth and tumorigenicity, i.e. cells are highly resistant to normal growth control signals (1,2). We have observed that growth inhibited 10T½ cells can produce a reversible growth inhibition of carcinogen transformed 10T½ cells (T10T½), that this inhibition requires cell/cell contact and is modulated by agents that influence cyclic nucleotide metabolism (2,3). In reconstruction experiments utilizing confluent monolayers of 10T½ seeded with T10T½ cells, growth of malignant colonies is conditional upon low serum concentration, which induces a low saturation density of 10T½ cells. Under conditions of low serum, cultures can be made non-permissive by addition of cyclic nucleotide phosphodiesterase (PDE) inhibitors. The most potent compound so far tested, Ro20-1724, induces a dose-dependent increase in cAMP content of 10T½ cells (50 fold at 10^{-4}M), and a dose-dependent decrease in size and number of T10T½ colonies. Drug treatment of T10T½ cells in the absence of 10T½ cells had no effect on cell growth and produced only a 3-fold elevation of cAMP. Treatment decreased levels of cGMP by 50% in both cell types and showed no dose-dependency. Cholera toxin enhanced the growth inhibition and cAMP elevation caused by Ro20-1724. Medium from non-permissive cultures was not growth inhibitory for T10T½ cells. Growth of a cell culture adapted line of Lewis Lung carcinoma is also slowed by co-incubation with 10T½ cells and PDE inhibitors, and furthermore, non-toxic doses of these agents in the mouse cause profound decreases in the incidence and size of lung metastases caused by this tumor (4). This decrease is not due to drug effects on cell seeding in the lungs, and as was found in vitro, growth inhibition is dependent upon continued drug administration. Cell cycle analysis has indicated a prolongation of G_1 in tumor cells metastatic to the lungs; no antiproliferative action on host tissues has so far been detected. PDE inhibitors are equally effective in tumor bearing "nude" mice as in conventional animals but have no effect in cells deficient in intracellular communication such as mouse L cells and the L1210 leukemia. A model will be presented for the direct communication of growth inhibitory signals between quiescent normal cells and transformed cells which can be modulated by cAMP.

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Carcinogenesis Overviews

430 THE ROLE OF DNA DAMAGE AND REPAIR IN MUTAGENESIS AND CARCINOGENESIS, John Cairns, Harvard School of Public Health, Boston, Mass. 02115.

One of the conspicuous features of cancer research is the wide variety of agents that have been shown to be capable of causing cancer. The list includes the various physical and chemical mutagens, a large number of viruses, certain non-mutagenic agents (such as asbestos and inert plastic films), the disordered growth that occurs when certain tissues are transplanted to particular sites in the body or are exposed to excessive hormonal stimulation, and lastly the simple act of overfeeding (which is carcinogenic for mice, in the sense that it is a prerequisite condition if they are to develop certain cancers). The precise causes of most human cancers are not known, although it is clear that there are external causative agents at work that are associated with life-style. We could conceivably identify these causes if we had a detailed understanding of the precise molecular biology of the common human cancers. Until that kind of information becomes available, we must be content with circumstantial evidence.

The available evidence suggests that local sequence changes, produced by conventional mutagens, are not making a major contribution to the incidence of human cancer; this is presumably in part because the various forms of DNA repair are very efficient and in part because such changes do not tend to produce drastic changes in phenotype. Major rearrangements of the genome seem to be much more important; unfortunately, although we know a lot about the production of local changes in sequence, much less in known about the factors that switch on the enzymes that catalyse genetic transpositions.

431 RELATIONSHIP BETWEEN CARCINOGENESIS AND TRANSFORMATION OF CELL CULTURES, Charles Heidelberger, Departments of Biochemistry, Pathology and the Comprehensive Cancer Center, University of Southern California, Los Angeles, CA, 90033

Carcinogenesis is studied in humans by epidemiologists, who have been excluded from this Symposium. They have shown, usually with a lag time of 20 years, that the majority of human cancers are environmentally caused by smoking, industrial exposures, radiations, and ill-defined "lifestyle" factors. On the whole, humans and primates appear to be quite resistant to carcinogenesis, whereas rodents are not. Human carcinogenesis probably proceeds through 5 stages (1). The study of carcinogenesis in animals is extremely complicated, since it is affected by the genetic, nutritional, hormonal, and immunological status of the host, and the pharmacokinetics, organotropism, metabolism, and chemical reactivity of the carcinogen. The process can be divided into at least the two stages of initiation and promotion; the latter may be of great importance in human carcinogenesis. Adequate carcinogenesis bioassays require hundreds of mice kept throughout their lifespan and are extremely expensive. Transformation in cell cultures is much simpler and more rapid, the environment and nutrition can be controlled, and it represents a relevant model system for studying mechanisms and for bioassay of carcinogens. Transformation involves a constellation of phenotypes; those most frequently noted are loss of contact inhibition of replication (piling-up), loss of anchorage independence (growth in soft agar), and ultimately acquisition of tumorigenicity. These occur in stages (2,3). The relevance of these model systems to carcinogenesis must continually be validated. The systems most frequently used for quantitative studies involve primary cultures of normal diploid Syrian hamster embryo fibroblasts (2,4), and permanent lines derived from mouse embryo cells, such as our C3H/10T1/2 cells (5); each has advantages and disadvantages. In general, there is a good correlation with various classes of physical and chemical carcinogens between their carcinogenic activity and their ability to transform cells, provided that suitable metabolic activation occurs or is provided externally. Epithelial cell systems have been difficult to quantitate. Human cells have been difficult to transform, but success has been achieved (6). Studies of mutagenesis and transformation in the same cells (7,8,9) suggest that mutagenesis, or a mutation-like phenomenon, is necessary but not sufficient for transformation.

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432 MODULATION OF NEOPLASTIC DEVELOPMENT DURING HEPATIC AND EPIDERMAL CARCINOGENESIS: A COMPARISON, Henry C. Pitot, McArdle Laboratory for Cancer Research, University of Wisconsin Medical School, Madison, Wisconsin 53706

The stages of initiation and promotion in epidermal carcinogenesis have been well characterized and defined during the past four decades since their initial description. Initiation in the skin is irreversible, exhibits a "memory", is more effective during specific times of the cell cycle and may be effected by agents incapable of promotion of the initiated cell (incomplete carcinogens). Promotion in skin is reversible, modulatable by environmental factors and results either from the action of a complete carcinogen which has promoting activity or by promoting agents which cannot initiate cells but may promote fortuitously initiated cells resulting from background effects.

During the last 5 years the stages of initiation and promotion during hepatocarcinogenesis have been clearly delineated. Their characteristics are essentially identical to those seen in skin with the additional finding that the immediate progeny of initiated cells can be identified by suitable histotechnologic methods. Such focal collections of cells known as enzyme-altered foci exhibit an enhancement of cell replication in response to hepatic promoting agents over that seen in noninitiated hepatic cells. Such foci exhibit an extensive degree of phenotypic heterogeneity quite analogous to the variety of phenotypes seen in primary and transplanted hepatocellular carcinomas. The cells of such initiated foci under appropriate conditions may be transplanted to new hosts further indicating their neoplastic potential even from an early period in their natural history. The reversibility of promotion during hepatocarcinogenesis can be demonstrated at low doses of the promoting agent. Thus the two stage concept of carcinogenesis has been extended to include both epidermal and hepatocarcinogenesis and likely represents a major component of the natural history of tumor development in many if not all histogenetic neoplastic types.

Carcinogen Metabolism: Pathways and Genetics

433 INDUCTION OF THE CYTOCHROME P-450 DEPENDENT OXIDATIVE METABOLISM OF AFLATOXIN B_1 IN THE MOUSE BY BUTYLATED HYDROXYANISOLE (BHA), Alexander W. Wood, Linda R. Wudl* and Richard L. Norman, Hoffmann-La Roche Inc., Nutley, NJ 07110

The ability of BHA to protect animals from chemically induced tumors has, in part, been ascribed to the capacity of the antioxidant to increase metabolic detoxification and/or decrease metabolic activation of the carcinogens. In the present study hepatic microsomes from untreated C57BL/6J mice and mice fed BHA (0.75% w/w of diet) were compared for their ability to metabolize aflatoxin B_1 to products mutagenic to Salmonella typhimurium. BHA feeding caused less than a 50% increase in hepatic cytochrome P-450 but increased aflatoxin induced mutations per nmol of hemoprotein 600 to 700% over the levels obtained with control microsomes. Microsomal incubations were analyzed by high pressure liquid chromatography to quantitate the formation of aflatoxin B_1 2,3-dihydrodiol, the spontaneous solvolysis product of the ultimate carcinogen of aflatoxin B_1, aflatoxin B_1 2,3-oxide. In accord with the mutagenicity studies BHA treatment induced aflatoxin B_1 2,3-dihydrodiol formation 600-700% over control levels. These results indicate that treatment of mice with BHA can markedly increase the oxidative metabolism of aflatoxin B_1 to its ultimately carcinogenic metabolite, and suggest that the anticarcinogenic effects of BHA reported with other carcinogens may not pertain with aflatoxin. Studies evaluating the effect of BHA on the metabolic activation of aflatoxin B_1 in vivo are in progress to establish the relevance of our in vitro observations.

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434 HUMAN CELL MUTAGENESIS: A QUANTITATIVE ASSAY AT THE HGPRT LOCUS EMPLOYING EMBRYONIC SKIN FIBROBLASTS AND RAT LIVER S9 HOMOGENATES. David J. Chen, Gary F. Strniste, and Richard T. Okinaka, Genetics Group, Los Alamos Scientific Laboratory, Los Alamos, New Mexico 87545

Most normal human fibroblasts grown in culture do not metabolize promutagens/procarcinogens. Thus screening assays employing human cells have only been successful for direct-acting chemical mutagens and various radiations. A mutation assay (HGPRT locus) employing a human embryonic skin fibroblast and a rat liver homogenate (S9) mixture is being developed. Three model promutagens, benzo(a)pyrene [B(a)P], 3-methylcholanthrene (3MC), and dimethylnitrosamine (DMN) have been utilized in these studies. In addition to discussing conditions for optimizing the response of this assay, our results indicate that at constant amount of S9 protein concentration, there exists a linear correlation between mutagenicity and dose. At 50% survival, the mutation frequencies induced by B(a)P and 3MC (5μg/mL) are 60 and 30 times the background mutation frequency, respectively. Similarly, at 50% survival, DMN (5mg/mL) induced 6-TG^R mutation frequencies are 25-fold over the background rate. The increase in cytotoxicity resulting from exposure of cells to these "activated" chemicals is linear with dose. At high S9 concentrations a deactivation or detoxification phenomenon occurs. However, the mutagenic potency of S9 activated chemicals when plotted as the number of induced mutations versus log survival is unaffected by the deactivating capacity of S9 proteins. This study demonstrates a quantitative mutation assay using an early passage human culture with an exogenous rat liver microsomal preparation providing activating enzymes. [This work was performed under the auspices of the United States Department of Energy.]

435 CONJUGATION AND DEACTIVATION OF BENZO(a)PYRENE BY ELEVATED LEVELS OF EXOGENOUS MICROSOMAL FRACTIONS. Richard T. Okinaka, David J. Chen, and Gary F. Strniste, Los Alamos Scientific Laboratories, Los Alamos, N.M. 87545

The potency of a given procarcinogen appears to be dependent not only on the activation process, but also on how effectively these chemicals can be deactivated by other cellular components. One such activation-deactivation phenomenon can be described in cultured Chinese hamster cells (line CHO) when the cells are treated with benzo(a)pyrene [B(a)P] and varying concentrations of liver homogenates (S9) from Aroclor-induced rats. Initial increases in S9 levels result in decreases in cell survival, with maximum cell killing observed at 0.5 mg/ml S9 proteins. Further increases in S9 concentrations, however, are followed by detoxification of the cytotoxic components. During this period mutation frequencies (HGPRT locus) rise from 1×10^{-5} to a maximum of 1.2×10^{-4} before dropping to 4×10^{-5} at higher S9 levels. Chromatographic separations using alumina columns and a 4-step gradient indicate that elevation in S9 concentrations results in significant increases in the level of conjugation of B(a)P to sulfates, glucuronic acid, and glutathione. These data suggest that S9 preparations possess the potential to remove cytotoxic and mutagenic species by conjugation mechanisms. Other experiments, however, reveal that the cytotoxic and mutagenic properties of S9 activated B(a)P can be nullified by the addition of competing nucleophiles such as bovine serum albumin--suggesting that detoxification observed with elevated S9 levels could be explained by an increase in non-specific binding sites for reactive B(a)P moities. It appears that benzo(a)-pyrene metabolites can be deactivated by S9 components by at least two distinct mechanisms. This work sponsored by the Department of Energy.

436 LIPOPROTEINS IN CELLULAR UPTAKE OF BENZO(A)PYRENE, Joyce F. Remsen and Rachel B. Shireman, University of Florida, Gainesville, Florida 32610

When a hydrophobic compound such as benzo(a)pyrene is added to plasma, it associates with the lipoproteins, especially the low density lipoprotein. A number of cell types have receptors on the plasma membrane for low density lipoproteins and these receptors are involved in cell internalization of the lipoprotein. We have investigated whether the low-density-lipoprotein-receptor pathway plays a major role in cell incorporation of benzo(a)-pyrene. Receptor-positive (WI-38) and receptor-negative (familial hypercholesterolemia) cells were compared for uptake of benzo(a)pyrene which was associated with low density lipoprotein or was added in acetone to medium with delipidated serum. The amount of benzo-(a)pyrene associated with the two cell types was the same whether we measured the kinetics, temperature dependence or concentration dependence. We, therefore, conclude that the relative amount of carcinogen taken into cells by the low density lipoprotein receptor pathway is insignificant compared with that which redistributes between the lipoprotein and the cell membrane. Post-treatment incubation with media containing one of several lipoproteins or albumin suggests the benzo(a)pyrene can also readily transfer out of the cells over a period of several hours.

437 COMPARISON OF BENZO(A)PYRENE METABOLISM BY TESTICULAR HOMOGENATE AND THE ISOLATED PERFUSED TESTIS OF RAT FOLLOWING 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN TREATMENT, Insu P. Lee and Junya Nagayama, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

In an effort to improve the extrapolation of laboratory data to man and better estimate the risk of potential human reproductive toxicity associated with environmental polycyclic aromatic hydrocarbons (PAH), benzo(a)pyrene (BP) metabolism in the testicular homogenate (TH) and in the isolated perfused testis (IPT) of the rat were analysed by HPLC and compared. The TH system metabolized BP 16 times the rate of the IPT. Furthermore, the pattern of BP metabolites in the IPT was significantly different from that in the TH. Thus, BP metabolism in these two systems are qualitatively and quantitatively different. Tetrachlorodibenzo-p-dioxin (TCDD) treatment not only increased the rate of BP metabolism 1.5 fold in both systems, it also increased the amount of BP metabolites in the organic phase as compared to the water soluble phase, suggesting TCDD induction of MFO enzymes favors increased formation of organic soluble BP metabolites. Distribution of BP metabolites indicated that only 9,10-diol and quinones readily reached an equilibrium between the perfusate and the testicular tissue compartment. The IPT system used in this study was metabolically active and offered a convenient method for studying the kinetics of overall toxication and detoxication reactions. In contrast to the in vitro TH system, the IPT retains the integrity of the multienzyme complex at different cellular locations in different testicular cell types. Therefore, it should more closely mimic in vivo testicular PAH metabolism and may provide the data necesssary for more valid toxicity assessment of gonadal toxicity.

438 REACTIVE ESTERS AS MODELS FOR THE ULTIMATE CARCINOGENS OF POLYCYCLIC HYDROCARBONS. J. W. Flesher and K. L. Sydnor, Univ. of Ky. Medical Center, Lexington, KY 40536

It is now generally accepted that polycyclic hydrocarbons must be metabolized to reactive intermediates capable of forming covalent bonds with cellular macromolecules in vivo in order to induce cancer. Professors James and Elizabeth Miller were the first to formulate a reactive ester hypothesis for polycyclic hydrocarbons (Proc. Soc. Exp. Bio. Med. 124:915-919, 1967). They postulated that 7-methylbenz(a)anthracene might be hydroxymethylated in the K-region (5 position) followed by further conversion to a reactive ester in vivo. We extended the work of Boyland, Sims and Huggins (Nature 207:816-817, 1965) and showed that 7-hydroxymethyl-12-methylbenz(a)anthracene, a metabolite of 7,12-dimethylbenz(a)anthracene, was a potent carcinogen. We also postulated that the first step in the metabolic activation of polycyclic hydrocarbons, which are not themselves reactive, is conversion to a meso-hydroxyalkyl metabolite. The second is the formation of a reactive ester bearing a good leaving group (Cancer Research 31:1951-1965, 1971). In accordance with this hypothesis synthetic acetate esters of meso-hydroxyalkyl metabolites are ultimate mutagens in the Ames assay and react with DNA in vitro (Res. Commun. Chem. Path. Pharm 22(2):345-355, 1978; Chem. Biol. Inter. 25:35-44, 1979). They are also potent carcinogens (Chem. Biol. Inter. 29:159-167, 1980). Furthermore, we found that ATP mediates the covalent binding of meso-hydroxyalkyl metabolites to DNA in vitro suggesting the formation of a reactive phosphate ester. The non-enzymatic ATP mediated binding of hydroxymethyl derivatives of aromatic hydrocarbons to DNA has been confirmed by Rogan, et al. (Chem. Biol. Interactions 31:51-63, 1980).

439 BENZO(A)PYRENE METABOLISM IN HUMAN MAMMARY EPITHELIAL AND FIBROBLASTIC CELLS, J. Bartley, J. Bartholomew, and M. Stampfer, Peralta Cancer Research Institute, Oakland, CA 94609, and Lawrence Berkeley Laboratory, Berkeley, CA 94720

We have utilized our newly developed system for growing human mammary epithelial cells (HMEC) for several passages in culture to study metabolism of the polycyclic aromatic hydrocarbon (PAH) benzo(a)pyrene (BaP). BaP was chosen because of its ubiquitous presence in the environment. MEC are particularly appropriate because mammary adenocarcinomas are preferentially induced by PAH in rodent systems. A comparison of the metabolic fate of BaP in normal epithelial and fibroblast cells from the same individuals has shown that HMEC utilize BaP much more readily and extensively than do fibroblasts. At 0.4 uM, HMEC had metabolized almost all the BaP by 24 hrs. Major metabolites in decreasing order were tetrols, water soluble conjugates, particularly with glutathione, and 7,8 and 9,10 diols which reached a maximum at 9 hrs. and decreased thereafter. Formation of BPDE II adducts with dG of DNA ceased after 6 hrs. BPDE I adducts were formed as long as substrate was available. Both BPDE-DNA adducts persisted for at least 72 hrs. after removal of BaP. In constrast, fibroblasts required 4 uM BaP for detectable metabolite production, characterized by monohydroxide and some 7,8-diol formation; DNA adducts were formed more slowly and to a lesser extent. Based on studies in model systems, the BaP metabolic pattern of HMEC is consistent with high susceptability to chemical carcinogenesis. The effect of lipids, especially those rich in polyunsaturated fatty acids, on BaP metabolism by HMEC will be reported. Supported by grants from American Cancer Society and from DOE.

440 METABOLISM OF MUTAGENIC FRACTIONS FROM COOKED GROUND BEEF, J.S. Felton, S.K. Healy, M. Knize, D.H. Stuermer, P.W. Berry, H. Timourian, F.T. Hatch, L.F. Bjeldanes and M. Morris, Biomedical Sciences Division, Lawrence Livermore National Laboratory and Dept. of Nutritional Sciences, Univ. of California, Livermore and Berkeley, CA 94550

Bacterial mutagens are formed during grilling of ground beef at 200°C (Commoner, et al., Pariza, et al., Springarn and Weisburger, this lab.). In the acetone extracted basic fraction a mean of 6300 Salmonella TA1538 rev. per 100 g fresh wt were obtained following activation with aroclor 1254 (PCB) induced rat liver 9000 x g supernatant (S9). S9 from PCB-treated hamster was twice as active as that from rat. Microsomal fractions from the inducible C57BL/6J mouse induced by PCB, 3-methylcholanthrene (3-MC) and β-naphthoflavone (BNF) showed revertant rates similar to the rat. In contrast, fractions from phenobarbital (PB), pregnenolone-16α-carbonitrile (PCN) and corn oil (CO) treated mice showed much lower metabolism. PB revertant levels were 20% of PCB, whereas PCN and CO microsomes were not active. Human liver microsomes from six individuals were very active. All activated the basic fraction significantly above the mouse and rat controls, and one human sample was 75% of the PCB treated rat. Species and inducer activation patterns for hamburger mutagens did not correlate with those of 2-acetylaminofluorene and 2-aminoanthracene nor with cytochrome P-450 levels and aryl hydrocarbon hydroxylase (AHH) activity. Hamburger mutagenicity did correlate with induction of two mouse P-450 proteins with molecular weights of 54,000 and 55,000 on SDS-polyacrylamide gel electrophoresis. Activation by mouse intestinal S9 was lower than liver S9 but was increased by induction of BNF (an analogue of natural flavonoids commonly found in foods) which also gave the highest AHH levels. Work spon. by U.S. DOE #W-7405-ENG-48 & NIEHS IGA #222YO1-ES-80038.

441 GENETIC ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBON ACTIVATION IN CULTURED HEPATOMA CELLS, Oliver Hankinson, University of California, Los Angeles, Ca 90024, and Catherine Legraverend, National Institute of Child Health and Human Development, Bethesda, Md 20205

The mouse line, Hepa-1, has high and stable activity of aryl hydrocarbon hydroxylase (AHH) and the enzyme is inducible by polycyclic aromatic hydrocarbons (PAH's) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). We isolated benzo(a)pyrene-resistant (BP^r) clones of this line. All BP^r clones had undetectable or reduced AHH activities. The properties of the clones indicate that they are mutational in origin. Thus they arose at a very low frequency (2×10^{-7} events per cell generation), their frequency was increased by the application of mutagens, and their phenotypes were stable. Thirty BP^r mutants have been combined in somatic-cell hybrids with the parental strain, Hepa-1. One is dominant, the others are recessive. The recessive mutants have also been hybridized with one another in various pair-wise combinations. The results so far show that the BP^r mutants belong to at least two complementation groups (and therefore that at least two different genes have been mutated). A representative of one complementation group lacks activity for a cytosolic TCDD-binding protein that is implicated in the PAH mediated induction of AHH. Another mutant has much reduced activity of UDP-glucuronosyltransferase activity (which is also inducible in these cells) as well as AHH. Future research will be directed towards ascertaining exactly how many complementation groups are represented among our mutants; determining the primary biochemical defect in each and its consequences on overall PAH metabolism; and isolating mutants of other enzymes of PAH metabolism. This research should provide considerable insight into the structure, function and regulation of these important enzymes.

442 LIPID MODULATION OF BENZO(a)PYRENE METABOLISM IN C3H 10T½ MOUSE EMBRYO FIBROBLASTS, Kathleen K. Dougherty and James C. Bartholomew, Lawrence Berkeley Laboratory, Berkeley, CA 94720

The polycyclic aromatic hydrocarbon, benzo(a)pyrene (B(a)P) is metabolized by C3H 10½ clone 8 mouse embryo fibroblasts and the quantity and kind of metabolites formed is effected by culture conditions. The cells have been cultured in different media (Minimal Essential Medium (MEM), Basal Medium Eagles (BME), and Dulbecco's Modified Eagles Medium supplemented with either fetal calf serum (FCS) or lipid depleted fetal calf serum (LDS). B(a)P metabolism has been measured by fluorescence emission spectra and radiometric analysis of ^3H-labeled metabolites separated by HPLC. Fluorescence emission data indicate that B(a)P is metabolized to different products when different culture conditions are used. Radiometric analysis of individual metabolites from cells cultured in BME or MEM indicate that cells cultured with FCS produced more water soluble metabolites than cells cultured with LDS. In addition, the percent water soluble metabolites increased with time of exposure to B(a)P when cells were cultured in FCS or LDS. Alumina Column analysis of cells cultured in MEM and FCS indicate that both glutathione and sulfate conjugates are formed. At present, there is no evidence of a glucuronide conjugate. Cells cultured with FCS also exhibited more overall metabolism of B(a)P than cells cultured with LDS. In conclusion, both serum composition and media selection have been shown to effect B(a)P metabolism in C3H 10½ clone 8 cells. (Supported by US DOE under contract no. W-7405-ENG-48.)

443 NOVEL VARIANTS IN BENZO(A)PYRENE METABOLISM: ISOLATION BY FLUORESCENCE-ACTIVATED CELL SORTING. James P. Whitlock, Jr. and Arthur G. Miller. Department of Pharmacology, Stanford University School of Medicine, Stanford, CA 94305.

We have used the fluorescence-activated cell sorter to study the metabolism of the carcinogenic polycyclic aromatic hydrocarbon, benzo(a)pyrene, in intact, viable cells. The approach involves exposing a cell population to low (nanomolar) concentrations of the carcinogen, and monitoring the disappearance of benzo(a)pyrene fluorescence as a function of time. The disappearance of fluorescence is accompanied by an increase in water-soluble benzo(a)pyrene metabolites and an increase in the amount of carcinogen covalently-bound to tissue macromolecules. The disappearance of fluorescence is inhibited by 7,8-benzoflavone at nanomolar concentrations. The disappearance of fluorescence is much more rapid in cells which have been pretreated with benz(a)anthracene or TCDD to induce benzo(a)pyrene metabolizing activity. Thus, the disappearance of benzo(a)pyrene fluorescence is due to metabolism. We have monitored the disappearance of benzo(a)pyrene fluorescence in hepatoma cells using the fluorescence activated cell sorter and have selected cells from which the fluorescence disappears either very rapidly or very slowly. In this way, we have been able to isolate stable cell variants with either markedly increased or markedly decreased ability to metabolize the carcinogen. This technique is generally applicable to many cell types and allows us to assess heterogeneity in benzo(a)pyrene-metabolizing activity within an individual or among different populations. The variants we have isolated will be useful in studying the biochemistry and genetics of microsomal enzyme activity.

444 RELATIONSHIP BETWEEN IN VITRO MUTAGENESIS AND IN VIVO CARCINOGENESIS BY NITROSAMINES, C.A. Jones and E. Huberman, Oak Ridge National Laboratory, Oak Ridge, TN 37830.

In vitro bioassays which measure the induction of different mutations may serve as a means of identifying potential carcinogenic agents. In order to facilitate meaningful risk assessment, we attempted to establish a quantitative relationship between the degree of tumor induction in experimental animals and mutagenic activity in cultured mammalian cells. This was accomplished by testing in a sensitive mammalian cell-mediated mutagenesis assay, twenty-seven nitrosamines for which a carcinogenic potency could also be established. In this cell-mediated assay, mutations for ouabain and 6-thioguanine resistance were measured in Chinese hamster V79 cells after the nitrosamines were metabolically activated by primary Fischer rat hepatocytes (Jones and Huberman, Cancer Res. 40: 406-411, 1980). The carcinogenic nitrosamines were found to be mutagenic in this assay whereas noncarcinogenic nitrosamines were inactive. An index for mutagenic potency (D_{10c}) was defined as the concentration of nitrosamine that yields a mutant frequency which is ten times higher than the spontaneous mutant frequency. An index for carcinogenicity was defined as a function of the dose of nitrosamine and time resulting in death from tumors of 50% of the exposed animals. Using these indices, we could establish a linear relationship between the degree of carcinogenicity and the degree of mutagenicity for this chemical class with a P value of 0.0001. It is suggested therefore that this hepatocyte-mediated assay is a sensitive and useful means of predicting the potential carcinogenic potency of nitrosamines and perhaps other classes of chemical carcinogens.
Research sponsored by EPA under Interagency Agreement 79-D-X0533, and the OHER, U.S. DOE, under contract W-7405-eng-26 with the Union Carbide Corp.

445 METABOLISM OF CHEMICAL CARCINOGENS BY CULTURED HUMAN TISSUES, Herman Autrup and Curtis C. Harris, Human Tissue Studies Section, National Cancer Institute, Bethesda, MD 20205

The metabolism of three different environmental carcinogens - benzo(a)pyrene (BP), aflatoxin B_1 (AFB) and N-nitrosodimethylamine (DMNA) - was studied in normal human bronchial, colonic, duodenal and esophageal tissues collected from the same patients (12 cases). The tissues were obtained from immediate autopsies and maintained as explant cultures. The activation of the carcinogens was determined by assaying their binding to cellular DNA and by measuring carcinogen metabolites released into the tissue culture media. The highest level of binding for all three carcinogens was seen in bronchus > esophagus = duodenum > transverse colon. A positive correlation in the activation of both DMNA and BP between bronchus and esophagus was seen, while no correlation was found between colon and the other organs. No correlation between the activation of BP and DMNA in the same organ was found. Quantitative, but not qualitative, differences in both the water-soluble and organo-soluble metabolites of BP were observed. The ratio of water-soluble to organo-soluble metabolites was significantly lower in the colon and duodenum than in the bronchus. Furthermore, these organs had a significantly lower level of glucuronide conjugates. These results indicate that the colon and duodenum may inefficiently remove toxic BP metabolites by conjugation pathways. Tetrols and diols were the major organo-soluble metabolites formed in all four organs, but the ratio of tetrols to diols was significantly higher in the bronchus than in the gastrointestinal tissues. The results indicate the complex quantitative and qualitative correlation with the metabolism of chemical carcinogens and different types of human tissues.

Quantitation of DNA Damage

446 DETERMINATION OF THE STRUCTURE OF THE ANTHRAMYCIN-DNA ADDUCT BY ^{1}H AND ^{13}C-NMR SPECTROSCOPY. John M. Ostrander*, Laurence H. Hurley*, M. S. Balakrishnan+ and Thomas Krugh+, *College of Pharmacy, University of Kentucky, Lexington, KY 40506 and +Department of Chemistry, University of Rochester, Rochester, NY 14627.

Anthramycin is an antitumor antibiotic produced by Streptomyces refuineus, which reacts with DNA in a highly specific and unique manner. Using ^{1}H-NMR on an anthramycin-dinucleotide adduct and ^{13}C-NMR on an anthramycin-calf thymus DNA adduct we have unequivocally determined the points of covalent attachment between anthramycin and deoxyguanine.

We had previously proposed [Hurley and Petrusek, Nature 282, 529 (1979) and Petrusek, et al., Biochemistry, in press (1980)] that anthramycin is attached through an inherently unstable aminal linkage (N-CH-N) to the 2-amino group of guanine in DNA, and the stability of the adduct is dependent upon secondary hydrogen bonding interactions between the drug and adjacent base pairs. Since denaturation leads to loss of the stabilizing hydrogen bonds and release of the drug, we were unable to isolate an anthramycin-deoxyguanosine adduct. We have therefore used a direct approach with ^{1}H and ^{13}C-NMR to elucidate the linkage points.

^{1}H-NMR on the anthramycin-dinucleotide pair adduct [anthramycin-p(dG·C)] showed that one of the 2-NH_2 protons of guanine is replaced upon formation of the adduct. ^{13}C-NMR on the anthramycin (enriched 35X specifically at C-11 with ^{13}C) calf thymus DNA adduct (55-200 nucleotide pair lengths) showed that the ^{13}C enriched C-11 position undergoes a 15.5 ppm upfield shift upon formation of the DNA adduct. Based upon model compounds this is precisely the chemical shift we would expect in conversion from a N-CH-O (anthramycin) to a N-CH-N linkage in the DNA adduct and confirms C-11 as the point of covalent attachment to DNA.

447 A NEW APPROACH FOR STUDYING THE MOLECULAR MECHANISMS OF MUTATION BY CARCINOGENS, Robert W. Chambers, New York University School of Medicine, New York, N. Y. 10016. When an electrophilic carcinogen reacts with DNA, several different kinds of covalent adducts are formed. At present, their is no direct evidence as to which of these lesions actually produce mutations. To deal with this problem, we have developed a site-specific mutagenesis system that utilizes an essential gene (gene G) of bacteriophage øX174 to examine the mutagenic properties of these different adducts located at a single, preselected site in gene G (O. S. Bhanot et al., (1979), J. Biol. Chem., 284 2684-12693). After transfection of spheroplasts with site-modified RF DNA, any mutant produced in vivo by replication and/or repair of the specific lesion can be rescued from the spheroplast lysate using a host cell carrying a functional copy of gene G on a plasmid. By isolating the DNA from individual mutants and sequencing it in the region that carried the site-specific covalent adduct in the parental DNA, it is possible to characterize the mutations exactly. Using this system, we have begun studies with several selected adducts to answer the following questions directly: (1) Which of the various adducts produced by a given carcinogen produce mutations? (2) What kind(s) of mutation do the different pre-mutational lesions produce? (3) What role do the various DNA repair systems play in producing these mutations?

448 INCREASED SENSITIVITY OF THE ALKALINE ELUTION TECHNIQUE TO DETECTION OF CHEMICAL DAMAGE IN THE PRESENCE OF 1-β-D-ARABINOFURANOSYLCYTOSINE (ARA-C). Jon I. Williams, Ph.D. Allied Chemical Corporation, Morristown, New Jersey 07960

Ara-C is a potent inhibitor of DNA polymerization in cultured mammalian cells, specifically preventing ligation of DNA strand breaks induced directly or indirectly by chemical damage and subsequent DNA repair. This suggests that repairable DNA damage events which do not produce DNA strand breaks per se can be identified by strand break analysis if ara-C is present during chemical treatment. This approach was tested with the DNA damaging agents 4-nitroquinoline-1-oxide (4NQO) and methyl methanesulfonate (MMS) in human cells using both the alkaline elution technique of Kohn et al. (1) and unscheduled DNA synthesis (UDS) as assays. The alkaline elution results indicate an approximate 40-fold increase in sensitivity for detecting 4NQO-induced damage and a 3-6 fold increase for detecting MMS damage, with linear dose-response functions for both chemicals over the concentrations studied. This level of detection provided increased sensitivity compared to that obtained in parallel UDS experiments with the additional feature of decreased labor. The differences in results with and without ara-C cotreatment and in normal or repair-deficient (Xeroderma Pigmentosum) human cells for a commercially important compound ("compound X") will be used to illustrate the value of this increased sensitivity: UDS results with compound X in both cell types were equivocal as were alkaline elution results in the absence of ara-C but a clear dose response was observed in normal human cells following cotreatment with ara-C and compound x. (1) K. W. Kohn et al., Biochemistry 15, 4629-4637 (1976).

449 COMPARATIVE STUDIES ON BINDING OF 2-ACETAMIDOFLUORENE AND 7-FLUORO-2-ACETAMIDOFLUORENE TO RAT LIVER NUCLEIC ACIDS, John D. Scribner, Gertrud Koponen, and Norma K. Scribner, Pacific Northwest Research Foundation, 1124 Columbia Street, Seattle, Washington, 98104.

The first proven adduct between DNA and an aromatic carcinogen was 8-(N-2-fluorenylacetamido)guanine. Numerous studies have concerned themselves with the physical chemistry and genetic significance of this lesion, yet it has been difficult to show its significance for hepatocarcinogenesis by 2-acetamidofluorene (AAF). We have examined the formation of this and other adducts in RNA and DNA of male and female Fischer rats, of female and male Sprague-Dawley rats, after injection of either AAF or 7-fluoro-AAF. While no 8-(N-2-fluorenylacetamido)guanosine (-deoxyguanosine) can be detected in enzymatic digests of RNA or DNA from female S/D rats given AAF, this adduct is clearly present in comparable digests obtained from Fischer female rats, although females of both origins have comparable sensitivity toward acetamidofluorene-induced hepatocarcinogenesis. No 8-(N-2-(7-fluoro-)fluorenylacetamido)guanosine (-deoxyguanosine) was detected in comparable digests from female S/D rats given 7-fluoro-AAF, although the latter is a powerful hepatocarcinogen in these rats. Feeding either AAF or 7-fluoro-AAF to S/D rats for 4 weeks before a single injection of tritiated 7-fluoro-AAF resulted in reduction of the guanine-C-8-amide adduct to undetectable levels in DNA, and to 10% of control levels in RNA. In contrast, non-acetylated adducts were present in much larger amounts from 7-fluoro-AAF than from AAF, and were increased greatly by feeding of 7-fluoro-AAF. These results suggest that the acetylated adduct has little significance for hepatocarcinogenesis by AAF.

450 IDENTIFICATION OF THE DNA ADDUCT FORMED FROM THE CARCINOGEN N-HYDROXY-2-ACETYLAMINOFLUORENE IN THE MAMMARY GLAND *IN VIVO*. Frederick A. Beland, Constance C. Weis, Nancy F. Fullerton and William T. Allaben, National Center for Toxicological Research, Jefferson, Arkansas 72079.

N-Hydroxy-2-acetylaminofluorene (N-hydroxy-AAF) is a mammary carcinogen in female rats. In this tissue, arylhydroxamic acid N,O-acyltransferase has been implicated with the metabolic activation of this carcinogen to a DNA binding species. To provide additional support for this hypothesis, we have examined the carcinogen-DNA adducts present in the mammary gland *in vivo* following administration of N-hydroxy-AAF. Adult female CD rats were treated, i.p., with 4.0 mg/kg [ring-^{3}H]-N-hydroxy-AAF (sp. act. 1.06 Ci/mmole). After 0.2, 1, 3, 14 and 28 days animals were sacrificed, the mammary glands dissected, and the epithelial cells isolated by the method of Moon *et al.* [J. Histochem. and Cytochem. 17, 182(1969)]. DNA was then isolated by solvent extraction and hydroxyapatite chromatography. After enzymatic hydrolysis of the DNA to deoxyribonucleosides, the adducts were analyzed by high pressure liquid chromatography. Only one adduct was detected and it was chromatographically identical to N-(deoxyguanosin-8-yl)-2-aminofluorene. The level of the adduct was maximum at 4 hours (1.5 adducts/10^{6} nucleotides) and then decreased, following first order kinetics($t_{\frac{1}{2}}$ = 14.4 days). Whether or not this loss represents DNA repair or cell turnover is currently being investigated. When [^{3}H]-N-hydroxy-AAF was incubated with the DNA in the presence of purified hepatic N,O-acyltransferase the same adduct was obtained as the only product. These results suggest the primary involvement of N,O-acyltransferase in the metabolic activation of N-hydroxy-AAF in mammary tissue.

451 ENZYMATIC REPAIR OF PYRIMIDINE PHOTODIMERS, William A. Haseltine and Lynn K. Gordon Sidney Farber Cancer Institute, Department of Pathology, Harvard Medical School, Boston, MA. 02115

The *M. luteus* and T4 pyrimidine dimer endonucleases contain two enzymatic activities, a pyrimidine dimer DNA glycosylase that cleaves the N-glycosyl bond between the 5' pyrimidine in the dimer and the corresponding sugar and an AP endonuclease that cleaves the phosphodiester bond 3' to the sugar. We have investigated the ability of this reaction product to serve as a substrate for other enzymes. These studies show that 1) the AP activity of the *M. luteus* and T4 endonucleases results in a 5' phosphoryl group at the cleavage site, 2) that the AP site at the 3' terminus is a substrate for the human placental AP endonuclease. This site is not a substrate for the polymerase activity or the 3'-5' exonuclease activities of *E. coli* DNA polymerase I DNA or the Klenow fragment of the enzyme. 3) That removal of the AP site with the AP endonuclease (but not with alkali) produces a 3' terminus that is a substrate for both activities of the DNA polymerase.

452 BINDING OF BENZO(a)PYRENE TO EPIDERMAL DNA AND RNA AS DETECTED BY SYNCHRONOUS LUMINESCENCE SPECTROMETRY AT 77 K. R. O. Rahn, S. S. Chang, J. M. Holland, T. J. Stephens, and L. H. Smith, Biology Division, Oak Ridge National Lab., Oak Ridge, TN 37830. The fluorescence associated with benzo(a)pyrene (BP) moieties covalently attached to the nucleic acid (DNA plus RNA) isolated from the epidermis of BP-treated mice was examined at 77 K by use of a photon-counting fluorimeter operating in the synchronous scanning mode. The nucleic acid was isolated and purified from treated mice, placed in 0.15 ml of aqueous solution, and frozen to 77 K. The excitation and emission wavelengths were scanned simultaneously with the monochromators set 28 nm apart. This setting coincides with the difference in wavelength between the excitation and emission maxima for the fluorescence of bound BP. A plot of the fluorescence intensity as a function of the exciting wavelength shows a peak at 300 nm, proportional to the DNA concentration, and a peak at 355 nm, proportional to the BP concentration. Therefore, the amount of BP per sample can be expressed relative to the amount of DNA present allowing comparisons to be made between samples varying in DNA concentration. Currently the level of detection is on the order of 1 BP residue per 200,000 bases in 40 μg of nucleic acid. This amount of nucleic acid can be isolated from the skin of a single mouse. The method described here is generally useful for detecting the binding to DNA of nonradioactive carcinogenic polynuclear aromatics which might occur following the topical application to animal skin in vivo of complex hydrocarbon mixtures such as synthetic fuels and crude oils. (Research sponsored by the Office of Health and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with the Union Carbide Corp.)

453 T4 UV-ENDONUCLEASE: GLYCOSYLASE AND AP-ENDONUCLEASE ACTIVITIES APPEAR TO BE COUPLED, Howard J. Edenberg, Department of Biochemistry, Indiana University School of Medicine, Indianapolis, Indiana 46223

Extracts from T4-infected Escherichia coli introduce single-strand breaks at the sites of pyrimidine dimers in ultraviolet-irradiated DNA, and have been widely used as a sensitive assay for pyrimidine dimers. The T4 UV-endonuclease preparation has recently been shown to consist of two activites: UV-DNA glycosylase and AP (apurinic/apyrimidinic) endonuclease, but the relationship between them remains unclear. We have examined the mechanism of action of T4 UV endonuclease. With limiting enzyme concentrations more single-strand breaks in UV-irradiated supercoiled SV40 DNA were detected in alkaline conditions than in neutral conditions, indicating that apyrimidinic sites were left in the DNA. Thus the two enzymatic activities do not act simultaneouly. Both the glycosylase activity and the AP-endonuclease activity were inhibited by adding depurinated DNA, which suggests a physical coupling of the two activities. Inhibition of AP-endonuclease by untreated DNA was also seen; this might reflect a low level of AP sites accumulated in the DNA used. When measuring endonuclease-sensitive sites with T4 UV-endonuclease preparations the addition of excess carrier DNA could therefore cause underestimates, especially if the analysis is carried out in neutral conditions.

454 CELLULAR DNA PRECURSOR TRIPHOSPHATES ARE TARGETS OF N-METHYL-N-NITROSOUREA(MNU), M.S. Baker and M.D. Topal, University of North Carolina Medical School, Chapel Hill, N.C. We recently found evidence that products of the reaction of MNU with dATP in vitro incorporate into DNA by pairing against G and C as well as T template residues (M.D. Topal et al. (1980) ICN-UCLA Symp. Mol. Cell. Biol. 19, 725-733) - behavior predicted to be mutagenic in vivo. Therefore, to assess the importance of DNA precursor pools as targets for chemical carcinogens in vivo, synchronous cultures of C3H10T1/2 cells were treated for 30 minutes during S phase with 0.03 mM [^{3}H]MNU. This dose was less than 30% toxic to the cells and had no inhibitory effect on DNA replication. Cells were lysed with triton and DNA precursor pools isolated free of other cellular components by chromatographic methods. DNA was also isolated. The distribution of methylated deoxynucleotides was found to be four times larger per cell in the pools than in the DNA. Since the concentration of deoxynucleotides in the pools was found to be 1000 fold less than in the DNA, this result suggests that deoxynucleotides in the pools are 4000 times more susceptible to methylation by MNU than are deoxynucleotides in the cell DNA. Further analysis showed that 64% of the methyl adducts in the pools were on nucleotides modified only on the base. (These base modified dNTPs represent 3.2% of the total dNTP present in the cell at the time of methylation.) The remaining 36% of the methyl adducts were on either the phosphate alone or both the phosphate and base. Experiments are underway to determine which sites of modification on the bases are critical to incorrect incorporation during DNA replication. Such incorporation may offer a direct explanation for the S phase dependence of neoplastic transformation of cells in culture. Supported in part by grants from the NIH (GM 24798, CA 28632, and CA 16086) to M.D.T.

455 CONFORMATIONAL CHANGES OF POLY(dG-dC).POLY(dG-dC) MODIFIED BY THE CARCINOGENS N-ACETOXY-N-ACETYL-2-AMINOFLUORENE AND N-HYDROXY-N-2-AMINOFLUORENE, Marc Leng and Evelyne Sage, Centre de Biophysique Moléculaire, Orléans, France.

Poly(dG-dC).poly(dG-dC) was modified by the reaction with N-acetoxy-N-acetyl-2-aminofluorene. The conformations of poly(dG-dC).poly(dG-dC) and of poly d(G-C)AAF were studied by circular dichroism under various experimental conditions. In 95 % ethanol, the two polynucleotides adopt the A form. In 3.9 M LiCl, the transition B form-C form is observed with poly(dG-dC). poly(dG-dC) but not with poly d(G-C)AAF. In low salt concentration, poly d(G-C)AAF behaves as a mixture of B and Z form, the relative percentage depending upon the amounts of modified bases. The percentage of Z form is decreased by addition of EDTA and is increased by addition of Mg^{++}. Spermine favors the Z form in modified and unmodified polynucleotides. No defect in the double helix of poly d(G-C)AAF is detected by S_I endonuclease.

Poly(dG-dC).poly(dG-dC) modified by N-hydroxy-N-2-aminofluorene can also adopt the Z form in high salt concentration but no Z form is detected in low salt concentration.

456 QUANTITATION OF CARCINOGEN-DNA MODIFICATION BY IMMUNOLOGICAL TECHNIQUES. Miriam C. Poirier and Stuart H. Yuspa, Laboratory of Experimental Pathology, National Cancer Institute, NIH, Bethesda, MD 20205.

Specific antibodies raised in rabbits against the major DNA adducts of 2-acetylaminofluorene (2-AAF) and benzo[a]pyrene (BP) have been employed in a variety of procedures (radioimmunoassay [RIA], microtiter plate assays, immunofluorescence and electron microscopy) to probe carcinogen-DNA interactions in cultured cells exposed to activated carcinogens and animals fed a procarcinogen. The aim of these studies is to explore the relationship of DNA modification and repair to mutagenesis and transformation and/or tumorigenesis. Studies with antibodies directed against the acetylated or deacetylated C-8 adducts of 2-AAF with deoxyguanosine have been useful to quantitate the relative proportion of each adduct by RIA. In a variety of cultured cells exposed to N-Ac-AAF, patterns of bound AAF-C-8 adducts have been determined and can be manipulated by pharmacologic techniques. In livers and kidneys of rats continuously fed 2AAF, the level of modification of DNA plateaus by 15 days and 7 days after removal of the carcinogen-containing diet 75% of total C-8 adduct remains on the DNA. An antibody to BP-modified DNA has been utilized to measure BP binding and repair in cultured epidermal cells by RIA, and to study cellular distribution of adduct by immunofluorescence. In addition, visualization of the adduct in DNA has been achieved by electron microscopy with a ferritin-tagged antibody. Microtiter plate assays have increased the limits of detectability of BP-DNA adducts to about 0.1 fmole/ μg DNA, a level which may permit determination of BP-DNA adducts in human tissues. Overall, antibodies are more specific, sensitive, and less costly than radiolabeled probes, and may yield new information on molecular mechanisms of carcinogenesis.

457 MECHANISM OF INDUCTION OF STRAND BREAKS IN DNA BY THE CARCINOGEN N-HYDROXY-1-NAPHTHYLAMINE. *F.F. Kadlubar, *W.B. Melchior,Jr., *T.J. Flammang, +C. Springgate, †A.J. Moss, Jr., and†W.A. Nagle. *Natl. Center for Tox. Res., Jefferson, AR, 72079, +Tulane Med. School, New Orleans, LA, 70112 and †V.A. Med. Center. Little Rock, AR, 72201.

The ultimate carcinogen, N-hydroxy-1-naphthylamine (N-HO-1-NA), has been shown to undergo an acid-catalyzed reaction with DNA by arylamination and arylation of the O^6 atom of guanine (>98% of the total covalently bound products). Molecular weight determinations of (N-HO-1-NA)-modified DNA by analytical ultracentrifugation after treatment with alkali, with formamide, or with S_1 nuclease indicated the presence of single strand breaks. Each treatment resulted in the same ratio of breaks/ adduct (one break/10-13 base adducts) over a wide range of modification (1-30 adducts/10^3 nucleotides). The presence of strand breaks was also confirmed by quantitation of 5'-termini with enzymatic [^{32}P] phosphate (P) incorporation experiments. Treatment of the modified DNA with purified hepatic apurinic acid endonuclease failed to increase the number of (N-HO-1-NA)-induced breaks. The kinetics of adduct formation and strand breakage were identical, each being nearly 1st order with respect to both N-HO-1-NA and DNA concentrations. Mg^{++} inhibited strand breakage and adduct formation and the degree of inhibition was inversely proportional to the DNA concentration. Since these data indicate that (N-HO-1-NA)-induced strand breakage does not arise from apurinic sites or alkali-labile phosphotriesters, we hypothesize that the DNA breaks result from an electrophilic attack of N-$(HO)_2^+$-1-NA on DNA-P, forming an electrophilic arylamine N-phosphotriester which can hydrolyze to cause strand breaks or decompose to an S_n1 reactant that binds to the O^6 of guanine.

458 THE DEVELOPMENT OF A RADIOIMMUNOASSAY FOR THE DETECTION OF PHOTOPRODUCTS IN MAMMALIAN CELL DNA, Judith M. Clarkson and David L. Mitchell, The University of Texas System Cancer Center, Science Park-Research Division, Smithville, TX 78957

Antiserum was prepared in rabbits against UV-irradiated DNA. Using a ^{125}I-labeled Protein A binding assay it was shown to be specific for UV-irradiated DNA, binding increasing as a function of logarithmic increases in dose. The same assay with UV-irradiated polydT: polydA established that the primary lesion recognized by the antibody is the thymine dimer. This antiserum was then used to develop a radioimmunoassay for the detection of photoproducts in DNA isolated from UV-irradiated mammalian cells. The technique monitors the competition between labeled UV-DNA and unlabeled sample DNA for antibody binding sites. The sensitivity of the assay was optimized by using ^{32}P-labeled plasmid DNA as competitive probe and is capable of detecting photodamage in cellular DNA at doses as low as 2.5 Jm^{-2}. The assay has been used to demonstrate DNA repair in both HeLa and CHO cells. The apparent removal of dimers from CHO cell DNA within three hours post-irradiation contrasts with data obtained with T_4 endonuclease. This discrepancy is currently under investigation. This loss of *antigenicity* with time post-irradiation was not apparent in DNA from UV-sensitive mutants of CHO cells, which are also unable to carry out unscheduled DNA synthesis.

459 SPECIFIC RECOGNITION AND CLEAVAGE OF APURINIC SITES IN DNA BY A TRYPTOPHAN CONTAINING PEPTIDE. J.J.Toulmé, T.Alev-Behmoaras and C.Hélène. Laboratoire de Biophysique - INSERM U.201, Muséum National d'Histoire Naturelle, 61 Rue Buffon 75005 PARIS - FRANCE

We previously demonstrated that binding of the tripeptide (Lys-Trp-Lys) to DNA involves two different types of complexes named C_1 and C_2. Whereas only electrostatic interactions take place in complex C_1, stacking between indole ring and nucleic acid bases was demonstrated to occur in complex C_2. The equilibrium is characterized by the association constant $K_2 = C_2$ (stacked)/C_2 (unstacked complex). We have shown that stacked complex is strongly favoured for head denatured DNA ($K_2 \simeq 5$) as compared to native DNA ($K_2 \simeq 0.3$).

We have studied the binding of Lys-Trp-Lys to DNA modified by dimethyl sulfate. The constant K_2 slightly increase with the percentage of methylated purines suggesting that these damages induce only limited destabilization of the DNA. Cleavage of the glycosylic bond of purines result in the formation of apurinic sites which are strong binding sites for the peptide. We calculated a K_2 value of about 200 for such a site. The overall association constant is increased by more than two orders of magnitude for an apurinic site as compared to a native one. The peptide Lys-Trp-Lys is therefore able to recognize apurinic sites through stacking interactions. Moreover we have shown that this peptide. Which bears three amino groups is able to cleave the ribosyl phosphate chain. This peptide therefore mimics an apurinic endonuclease : it exhibits both a specificity of recognition and an endonucleolytic activity with respect to apurinic sites.

460 **AN IN VIVO BIOASSAY FOR DNA DAMAGE** Cheng M. Su, Douglas E. Brash, Steven M. D'Ambrosio and Ronald W. Hart. Depts. of Radiology and Pharmacology, The Ohio State University, Columbus, Ohio 43210.

Most carcinogens and mutagens produce DNA damage. We have developed a quantitative and sensitive in vivo bioassay for DNA damage in target and non-target organs, sensitive and resistant strains. The assay is based on detecting alkaline sensitive sites in non-radiolabelled DNA following sedimentation in alkaline sucrose gradients. The potent carcinogens ethylnitrosourea (ENU) and diethylnitrosamine (DEN) and their non-carcinogenic analogs benzylnitrosourea (BNU) and dibenzylnitrosamine (DBN) were injected into 30-day old female Sprague-Dawley rats. Nuclei were prepared from brain, liver and kidney and sedimented through alkaline sucrose gradients. The number of alkali-labile sites (phophotriesters) were determined at 1 hr. and 7-day post-injection. The target organ generally showed a proportionally higher amount of residual DNA damage over a 7-day period. The number of persistent lesions in DNA appeared to correlate with the degree of the potency of the carcinogen, since non-carcinogenic analogs displayed both a lower level of DNA damage and a faster rate of removal of the damage than the carcinogen. This assay, thus for the first time correlates in vivo the extent of DNA damage induced by alkylators and repair of the damage to organ susceptibility. Supported by USEPA Grant No. R805337.

461 **QUANTITATION OF DNA DAMAGE INDUCED BY ALKYLATING AGENTS AND DNA REPAIR IN VIVO** Altaf A. Wani, Ronald W. Hart, Steven M. D'Ambrosio. Depts. of Radiology, Pharmacology, The College of Medicine, The Ohio State University, Columbus, Ohio 43210.

Specific alkylation lesions induced by methyl methane sulfonate in the DNA of liver and brain of Sprague-Dawley female rats at 30 day of age were determined. Bases alkylated at the N_3 position of adenine were converted to apurinic sites by N_3-alkyladenine DNA glycosylase from Micrococcus luteus. The total number of alkylated bases (N_3A and N_7G) was estimated by heat depurination at 70°C for 30 min. Both enzyme and heat induced apurinic sites were detected by conversion to single-strand breaks upon incubation with 0.1 M NaOH followed by centrifugation in alkaline sucrose gradients. The DNA was detected using a fluoro-metric method. Both brain and liver showed a dose dependent linear increase in the number of DNA strand breaks. Liver showed a greater repair of N_3-adenine in comparison to brain, while the repair of N_7-guanine in both tissues was slower. We also studied the formation and repair of similar damage induced by methylnitrosourea and ethylnitrosourea which also alkylate the phosphodiester backbone. The technique does not require the use of radioactively labelled reagents and permits the quantitation of apurinic sites in presence of phosphotriesters. Supported by USEPA Grant No. R805337.

462 AN ENDONUCLEASE PREPARATION FROM HELA CELL NUCLEI STIMULATES DNA REPAIR IN NUCLEI FROM UV-IRRADIATED WI-38 FIBROBLASTS. Bradford S. Fansler, Thomas M. Vollberg and Steven H. Robison. Dept. of Pathology, Thomas Jefferson Univ., Phila., Pa. 19107.

We have developed a method for measuring DNA excision-repair synthesis in nuclei isolated from human cultured cells, WI-38 cells which had grown to confluence and ceased DNA replication were irradiated with UV light and the nuclei isolated. Repair synthesis was determined by ^{3}H-dTMP incorporation into damaged nuclei compared to undamaged nuclei. To confirm the incorporation as repair synthesis BrdUTP was added to the reaction mixture and density gradient analysis demonstrated that synthesis was in parental DNA. Isolated nuclei are advantageous for these studies since they maintain the integrity of the chromatin and nuclear structure, but are also permeable to the direct precursors of DNA repair synthesis, the dNTPs, and can take up repair enzymes. As a source for human DNases, nuclei isolated from HeLa cells were extracted with an isotonic NaCl-buffer solution to solubilize the nucleoplasmic enzymes. The remaining chromatin pellet was treated with a 1.0M NaCl-buffer solution to extract the chromatin-bound enzymes. This high-salt extract contains primarily endonucleases activity with small amounts of DNA polymerase and exonuclease activities. The polymerase and exonuclease were removed by treatment with CM-Sephadex, leaving nearly all of the endonuclease activity. Aliquots of this crude extract were added to the nuclear DNA repair system. This caused a doubling of ^{3}H-dTMP incorporation over UV treated nuclei without added DNase and a four-fold increase over unirradiated control nuclei. The UV dimer specific endonuclease from M. luteus causes a large increase of repair synthesis in UV treated nuclei. We are attempting purification of the UV-endonuclease and have found a UV-specific activity after DEAE-cellulose chromatography.

463 (^{32}P)BASE ANALYSIS OF DNA CONTAINING MODIFIED NUCLEOTIDES, Kurt Randerath, Ramesh C. Gupta and Marat V. Reddy, Baylor College of Medicine, Houston, TX. 77030.

Base composition analysis of carcinogen-modified DNA usually entails the reaction of DNA in vitro or in vivo with radioactive carcinogens followed by appropriate digestion of DNA and chromatographic analysis. Difficulties of this approach, particularly when applied in vivo, include requirement of large amounts of radioactive carcinogens, high cost, and low sensitivity. An alternative method for DNA base composition analysis, which does not require radioactive carcinogens, is being developed in our laboratory. Carcinogen-modified DNA is enzymatically digested to a mixture of deoxyribonucleoside 3'-monophosphates. Subsequent enzymatic incorporation of ^{32}P into these compounds by the T_4 polynucleotide kinase-catalyzed reaction yields (5'-^{32}P)deoxyribonucleoside 3',5'-bisphosphates which are subsequently resolved by thin-layer chromatography and assayed by counting. Conditions are being standardized for both digestion and quantitative analysis to make the procedure applicable to the determination of natural and chemically modified constituents of DNA.

464 THE USE OF A DNA BINDING PROTEIN TO DETECT BASE MISMATCH DAMAGE IN ALKYLATED DNA
Ross S. Feldberg and Saul A. Slapikoff, Tufts University, Department of Biology, Medford, Mass 02155

A DNA binding protein with a broad specificity for monofunctional base damage (Nuc. Acids Res. (1980) 8, 1133-1143) has been employed as a sensitive probe to measure the introduction of damage into DNA by alkylating agents. Comparison of N-methyl-N-nitrosourea (MNU) and dimethylsulfate (DMS) revealed a dose-dependent introduction of protein-recognizable damage with MNU, but no such damage with DMS at equivalent levels of alkylation. A major difference between these two agents is that MNU will introduce a significant level of alkylation at the O-6 atom of guanine whereas DMS yields little or no alkylation at this site. Since O-6 guanine alkylation would result in the disruption of guanine-cytosine base pairing, these results support our hypothesis that the human damage-specific DNA binding protein is able to recognize base mismatches in DNA.

465 DNA ADDUCTS FORMED BY 1'-HYDROXYSAFROLE IN VIVO AND BY 1'-ACETOXYSAFROLE IN VITRO.
D.H.Phillips, J.A.Miller, E.C.Miller and B.Adams, Dept. of Biological Sciences, Stanford Univ., Stanford, CA 94305, and McArdle Lab. for Cancer Research and Dept. of Chemistry, Univ. of Wisconsin, Madison, WI 53706.

Comparison by high pressure liquid chromatography of the nucleoside adducts obtained from hepatic DNA of adult female mice treated with [2',3'-^{3}H]1'-hydroxysafrole (1'-HO-S) with those formed by reaction of deoxyribonucleosides with 1'-acetoxy-S, 1'-HO-S-2',3'-oxide and 1'-oxo-S indicated that the four in vivo adducts studied were derived from an ester of 1'-HO-S. Three of the four in vivo adducts comigrated with products of the reaction of 1'-acetoxy-S with deoxyguanosine (dGuo), and the fourth adduct comigrated with the major product of this ester reacted with deoxyadenosine (dAdo). Synthetic samples of the major dGuo adduct and the dAdo adduct were characterized from their NMR spectra as N^2-(trans-isosafrol-3'-yl)-deoxyguanosine and N^6-(trans-isosafrol-3'-yl)-deoxyadenosine, respectively. The other two dGuo adducts also appeared to be substituted on the 2-amino group of guanine, on the basis of their pH-partition coefficient patterns and their retention of ^{3}H from [8-^{3}H]dGuo. Binding of 1'-HO-S to hepatic DNA in mice reached a maximum within 24 hrs of treatment. The levels of bound adducts decreased rapidly between 1 and 3 days after treatment, but approximately 15% of each adduct persisted for at least 20 days. Comparative studies are in progress on the response in cultured human cells to DNA damage by 1'-acetoxy-S.

466 DNA-PROTEIN CROSSLINKING BY CHROMIUM SALTS, A. J. Fornace, Jr. and C. C. Harris,
Human Tissue Studies Section, National Cancer Institute, Bethesda, MD 20205

Chromate salts are potent carcinogens implicated in the etiology of lung cancer in chromate workers. Previous studies have indicated that the hexavalent form of chromium crosses the cell membrane and is then reduced to the trivalent form. Since the trivalent form has a strong tendency to form coordination complexes with a coordination number of 6 and a very slow rate of ligand exchange, we investigated the possibility that chromium salts could produce crosslinking of DNA to other macromolecules. When cells were treated with K_2CrO_4, high levels of DNA-protein crosslinks (DPC) were detected by alkaline elution. In human fibroblasts, a 2h exposure to 200 μM K_2CrO_4 at 37° induced the same level of DNA-protein crosslinks as did 100 μM trans-Pt(II) diamminedichloride--a potent DNA-protein crosslinking agent. K_2CrO_4 induced similar levels of DPC in human fibroblasts, mouse L1210 cells, and human bronchial epithelial cells--the apparent cells of origin of the malignancies reported in chromate workers. The level of DPC was proportional to the concentration of chromate used, and appeared to be persistent since no removal was seen after 12h of repair incubation. Low levels of DNA single strand breaks were also induced in cells by chromate but were quickly rejoined. The active form of chromium appears to be the trivalent since chromic but not chromate salts induced DPC in isolated nuclei. Chromic salts also produced crosslinking between DNA and protein in solution while the hexavalent form was inactive. A reasonable interpretation of these results is that the hexavalent form crosses the cell membrane, is reduced to the trivalent form, and then forms stable coordination complexes with 2 or more macromolecules including DNA with protein.

467 A New Method for Determining the Location of Chemically Stable Lesions in DNA Fragments of a Defined Sequence, Brigitte Royer-Pokora and William A. Haseltine, Sidney Farber Cancer Institute, Harvard Medical School, Boston, MA. 02115

Exonuclease III was used for the detection of chemically stable lesions in DNA fragments of a defined sequence. Exonuclease III degrades double stranded DNA almost to completion. DNA containing pyrimidine dimers stops the action of Exo III. Comparing the fragments obtained after Exo III digestion with fragments obtained in a sequencing reaction reveals that Exo III stops one base away from the pyrimidine dimers. Comparison of the bands obtained after Exo III digestion with those obtained with the pyrimidine dimer specific corendonuclease showed that both enzymes quantitatively recognize pyrimidine dimers. This method was also applied to the analysis of modifications created by cis- and trans-Dichlorodiammineplatinum. Only the cis-isomer is active as an antitumor drug. It is known that both interact with DNA in a chemically stable fashion. We could detect an inhibition of Exonuclease III reaction at specific sites in DNA. Some differences were obtained for the cis- and trans compounds. All Exonuclease III stop sites found were in the vicinity of guanosine bases. It is known that guanosine is the preferential binding site for both platinum compounds. Therefore Exonuclease III can be used to determine the location of chemically stable lesions in DNA.

468 A Unified Mechanism of Interaction Between Nucleic Acids and Alkyl Nitrosoureas - A Hypothesis Supported by Experimental Data. J. Krepinsky[a], J. P. Carver[a], P.M. Rao[b] and D.S.R. Sarma[b], Departments of [a]Medical Genetics and [b]Pathology, University of Toronto, Toronto, Ontario, Canada M5S 1A8. The interaction at the molecular level between informational macromolecules such as DNA and chemical mutagens may be a crucial step in mutagenesis and carcinogenesis. It has been shown that carcinogenalkylated DNA can be analysed by NMR spectroscopy without degrading it[1]. An intermediate alkyl carbocation is generally assumed to be responsible for the reaction with DNA. However, N-methyl-N-nitrosourea (NMU) generates under a variety of conditions also isocyanic acid derivatives[2]. Therefore, we searched for products of DNA-NMU interaction between rat liver DNA and ^{13}C labelled NMU ($ON\text{-}H(CH_3)\text{-}^{13}CONH_2$) by NMR spectroscopy at 90 MHz in D_2O. We found the expected signal of a sp^2 carbon at δ=162.5 ppm assigned to a transformed cytosine ($-NH_2 \rightarrow -NH^{13}CONH_2$) accompanied by a much stronger signal of a sp^3 carbon at δ=60.4 ppm which is most likely due to a hemiketal type of bonding. Such a hemiketal structure might be part of an acid labile dimer involving a previously alkylated base. Since a transient adduct is formed between DNA and nitroso compounds[3] it appears likely that a DNA autocatalysed "push-pull"concerted reaction mechanism might better explain the regioselectivity of NMU action than the mechanism involving a naked carbocation. References: 1, J. Krepinsky et al., Chem. Biol. Interactions 27 (1979) 381; 2, A.M. Serebryanyi and R.M. Mnakatsanyan, Dokl. Akad. Sci. USSR 199 (1971) 657; 3, M. Jamaluddin et al., PNAS, submitted.

469 Correlation between Biphasic Production of O^6-Methylguanine and Induction of Mutations by N-Nitroso-N-Methylurea in unadapted Salmonella Typhimurium - Joseph B. Guttenplan and Sam Milstein, New York University, D.C. New York, N.Y. 10010

Mutagenesis induced by treatment of Salmonella typhimurium with N-nitroso-N-methylurea in buffer is biphasic with a low sensitivity range at low doses where little mutagenesis occurs followed by a high sensitivity range whose onset begins after an apparent threshold dose has been exceeded. Levels of O^6-methylguanine in the DNA extracted from the bacteria follow the same dose-response curve suggesting a dependency of mutagenesis on O^6-methylguanine. In contrast, levels of 7-methylguanine in the DNA increased near linearly with dose. The lack of O^6-methylguanine at lower doses of NMU is attributed to a saturable, constitutative repair activity in the bacteria. An attempt to observe the removal of O^6-methylguanine from the bacteria after exposure to a short challenge dose of NMU followed by a subsequent incubation was unsuccessful, probably because all the repair occured within time necessary to treat and lyse the cells. From the dose-response curves for mutagenesis and O^6-methylguanine formation it was calculated that the equivalent of 23 umole of O^6- methylguanine/mol guanine were removed or possibly not formed before appreciable mutagenesis occured. It was also calculated that each umole of O^6-methylguanine formed per mol nucleotide corresponded to a reversion frequency of 2.5×10^7.

470 THE ORGANIZATION AND EXPRESSION OF VIRUS-LIKE SEQUENCES IN TRANSFORMED AND NON-TRANSFORMED MOUSE CELLS, Michael G. Courtney, Lucy J. Schmidt and Michael J. Getz, Cell Biology, Mayo Clinic/Foundation, Rochester, MN 55901

Mouse virus-like (VL) 30S RNA is thought to be a defective retroviral genome, possibly a progenitor virus, and studies have implied that it can play some role in cell transformation. We have isolated a clone (BVL-1) which contains a 13 kb fragment of mouse DNA carrying a complete 5.5 kb VL gene from a Charon 4A BALB/c mouse genomic library. Using fragments derived from the VL coding region as probe, Southern Blots of EcoRI-cut DNA from various mouse cell lines display a strain specific pattern of hybridization indicating that the sequences are capable either of rearrangement or of integration by some retroviral-like mechanism. Transcription mapping experiments show that VL-related RNA is not present at detectable levels in polysomal poly(A)$^+$ RNA from mouse AKR-2B cells, but is present in polysomal poly(A)$^+$ RNA from a chemically transformed derivative cell line, AKR-MCA. Thus, the presence of VL-RNA is correlated with the relative abilities of cells to grow in soft agar and to form tumors when injected into suitable hosts. Further experiments show that mouse DNA sequences which flank the VL DNA sequence in BVL-1 are expressed in both AKR-2B and AKR-MCA cells as polyadenylated polysomal RNA suggesting that the VL gene in BVL-1 is integrated immediately adjacent to a genetically active site in the mouse genome.

471 THE PHOTOREVERSAL-DEPENDENT RELEASE OF FREE THYMINE FROM UV-IRRADIATED Escherichia coli, Eric H. Radany and Errol C. Friedberg, Stanford University, Stanford, CA 94305

Previous studies from this laboratory have demonstrated the action of a pyrimidine dimer (PD) -DNA glycosylase coded by the denV gene of bacteriophage T4. (1,2) This demonstration is based on the release of free thymine (by direct photoreversal of dimers) from DNA in which glycosylic bonds linking thymine in pyrimidine dimers to the deoxyribose-phosphate backbone, have been enzymatically hydrolyzed. In the present studies we have investigated the possible presence of a PD-DNA glycosylase activity in uninfected E. coli. Cells were prelabeled in DNA with ^{3}H-thymidine, exposed to UV radiation and incubated at 37°C. The acid-soluble fraction (in which excised thymine-containing pyrimidine dimers are present) was extracted and exposed to photoreversing radiation or not. In repeated experiments the photoreversal-dependent release of free thymine has been detected. This result is not observed with fractions prepared from unirradiated cells or from a strain of E. coli defective in pyrimidine dimer excision. Quantitative experiments designed to determine the role of this phenomenon in the molecular mechanism(s) of pyrimidine dimer excision in E. coli are currently in progress.

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2. Radany, E.H., Love, J.D., and Friedberg, E.C. In "Chromosome Damage and Repair", (eds. E. Seeberg and K. Kleppe) Plenum Press, NY (In press).

472 EVIDENCE THAT THE PRODUCT OF THE V GENE OF BACTERIOPHAGE T4 CODES FOR BOTH PYRIMIDINE DIMER GLYCOSYLASE AND AP ENDONUCLEASE ACTIVITIES. Huber R. Warner and Lise M. Christensen, University of Minnesota, St. Paul, MN 55108

It has recently been discovered that the UV-specific endonuclease induced by bacteriophage T4 incises UV-irradiated DNA by a two-step mechanism. The glycosylic bond between the pyrimidine and the deoxyribose at the 5' end of the dimer is first cleaved to produce an apyrimidinic (AP) site; the DNA strand containing the dimer is then nicked on the 3' side of this AP site. Seawell et al. (J. Virology 35:790-797 (1980)) have demonstrated that the T4 V gene codes for the glycosylase activity. We have obtained three kinds of evidence that this V gene product also contains the AP endonuclease activity. After chromatography on DEAE-cellulose, the two activities co-purify during phosphocellulose and Sephadex G-100 chromatography, with constant ratio of activities across the activity peaks. On Sephadex G-100, the molecular weights of the two activities can differ by no more than 2,500 daltons. When an extract of cells infected with a T4 V mutant is purified exactly as an extract of cells infected with T4 V$^+$, neither glycosylase nor AP endonuclease activity can be detected in the normal position for elution of the T4 UV endonuclease activity. Lastly, glycosylase and AP endonuclease activities are lost in parallel during incubation of the enzyme at 46°C. These results strongly suggest that both activities are contained in the same polypeptide, which is the gene product of the V gene.

473 Immunological Detection of Carcinogen Modified DNA Fragments Separated by Agarose Gel Electrophoresis. M. M. Seidman, H. STor, and M. Bustin, NCI, NIH, Bethesda, MD 20205

Antibodies specific for trans-7,8 dihydrobenzo[ ]pyrene-7,8-diol-9,10-epoxide-anti (BPDEI) modified DNA have been prepared. We have developed a technique in which these antibodies are used to detect BPDEI adducts on restriction fragments of carcinogen modified viral and plasmid DNA. The modified DNA is digested with appropriate enzymes; the fragments resolved by agarose gel electrophoresis and transferred to activated amino benzyloxymethyl cellulose (ABM) paper. The paper is incubated with antibody and then with ^{125}I Protein A. Fragments carrying covalently bound BPDEI can be detected by autoradiography. The amount of antibody Protein A bound is proportional to the amount of carcinogen bound and there is no reaction with unmodified DNA. Less than one adduct per DNA molecule can be detected with sub-microgram amounts of viral or plasmid DNA. We have used the technique to localize carcinogen bound to SV40 DNA in-vivo and in-vitro.

474 THE USE OF FLUORESCENT PROBES TO INVESTIGATE CONFORMATIONAL CHANGES IN NUCLEOSOMES INDUCED BY ANTI-TUMOR DRUGS AND CARCINOGENS, Michael Arquilla, David Gross and Henry Simpkins, University of California Irvine, Irvine California 92715

Terbium a rare earth lanthanide has been shown to react with non-structured guanine and xanthine residues to produce strong fluorescence enhancement. This enhancement is dependent on the number of G residues in the polymer chain as the shape of the binding curve moved from sigmoidal to hyperbolic as the chain length increased, i.e., non-cooperative interactions predominated at longer chain lengths. It has also been found that if the guanine ring was perturbed by the addition or substraction of various electron donating groups, then the enhancement could be increased or diminished. Since some anti-tumor drugs and carcinogens appear to act on the G residues in DNA, it was postulated that this probe might provide a sensitive probe for this interaction. We therefore have studied the interaction of cis-dichlorodiamine platinum (DDP) with DNA, RNA and chromatin. Our initial experiments show that the reaction of poly G, rRNA and tRNA resulted in a diminished terbium fluorescence in contrast to DNA where an increase was observed. Thus, the interaction of the anti-tumor drug with the G residues in DNA results in the G residues becoming less hydrogen bonded and more accessible to the probe, resulting in increased fluorescence. The effect correlates well with DNA G+C content. In addition, a fluorescent probe which specifically labels histone H3 was used to investigate the interaction of cis-DDP with nucleosomes and it was found that cis-DDP produced marked decreases in fluorescence enhancement following incubation of the nucleosomes with the anti-tumor drug. The diminution was more apparent with the trans (inactive) drug and the fluorescence emission spectra showed a spectral change, probably due to interaction between the labelled H3 residues.

475 ALTERATIONS IN GENE EXPRESSION BY CHEMICAL CARCINOGENS, Thomas Meehan, Michigan Molecular Institute, Midland, Michigan 48640

We are studying carcinogen - DNA interactions as a model for carcinogenesis. Carcinogens are generally mutagenic and their interactions with DNA may be important in describing the biological properties of these materials. Our studies on the activated form of benzo[a]pyrene (anti BaP diol epoxide) have shown that this carcinogen undergoes a number of specific interactions with DNA. Among the large number of nucleophilic sites in DNA BaP diol epoxide alkylates only the exocyclic amino groups of dG, dA, and dC. Another level of specificity that we have discovered is stereoselective covalent binding. The anti-BaP diol epoxide when chemically synthesized exists as a racemic mixture of enantiomers. One of these enantiomers covalently binds more efficiently (in ratios of 20:1) to double stranded DNA, while both bind to the same extent to single stranded DNA. The stereoselective covalent binding is a result of asymmetries in the DNA double helix. In addition to these results we have shown that the BaP diol epoxide undergoes physical binding by an intercalation mode into DNA. A number of intercalating agents are sequence specific and we are at present investigating this possibility for the BaP diol epoxide. The specificity involved in these reactions, in addition to the biological potency of BaP diol epoxide, suggest that the hydrocarbon may bind to and result in altered expression of specific genes. Since adduct analysis may not be capable of distinguishing which mutagenic events are replicated and transcribed we have begun an investigation into the molecular events which result after DNA damage.

476 A SENSITIVE DNA REPAIR ASSAY IN HUMAN TISSUE MAY BE EMPLOYED TO MEASURE THE IN VITRO EFFECTS OF MUTAGEN BINDING TO SERUM COMPONENTS, David L. Busbee, Patrick W. Rankin, D. Michael Payne and Daniel W. Jasheway, North Texas State University, Denton, TX 76203

Human lymphocytes were exposed in vitro to increasing concentrations of 7,8-diol-9,10-epoxy-benzo(a)pyrene (BPDE), 7-bromomethylbenzanthracene (BrBA), or N-methyl-N'-nitro-N-nitroso-guanidine (MNNG). Unscheduled DNA synthesis was assessed as a measure of ^{3}H-thymidine incorporated into cellular DNA in mutagen treated, non-mitogen stimulated, lymphocytes, as was seen to be linear for BPDE between 0.002 µg/ml and 0.1 µg/ml. UDS initiated by BPDE at 0.002 µg/ml was 250% above the control level. Above a mutagen concentration of 0.1 µg/ml the level of ^{3}H-thymidine incorporation began to decrease, dropping to the control level with the increase in BPDE concentration to 10 µg/ml. Lymphocytes were viable at that mutagen level, as measured by trypan blue exclusion and poly(ADP-ribose)polymerase activity. A direct correlation was seen between the decrease in repair incorporation of ^{3}H-thymidine and the capacity to engage in mitogen stimulated blastogenesis. As long as repair increased linearly along the log mutagen concentration plot blastogenesis was not impaired. Differing lots of fetal calf serum were seen to differentially bind polynuclear aromatic hydrocarbon carcinogens, changing their pharmacologically available levels, and affecting cellular response to the carcinogens. Several serum components bind ^{3}H-benzo(a)pyrene, including low density lipoprotein and high density lipoprotein. Data suggest that differences in ^{3}H-benzo(a)pyrene binding to a high molecular weight fraction of HDL may be critical in determing the available mutagen dose presented to the cell in culture.

477 AN IN VITRO SYSTEM FOR REPAIR OF DNA DAMAGE USING PURIFIED PROTEINS, Paul K. Small, Diane C. Rein, Dale W. Mosbaugh and Ralph R. Meyer, University of Cincinnati, Cincinnati, Ohio 45221

The types of damage to DNA caused by various mutagenic and carcinogenic agents have been well documented. Much less, however, is known of the molecular mechanisms for repair of such damage. Our goal is to establish an in vitro repair system using purified enzymes. DNA polymerase-β, the polymerase thought to be responsible for repair synthesis, has been purified to homogeneity from the Novikoff hepatoma. Concurrently, DNase V, a 3'→5' and 5'→3' bidirectional exonuclease active on double-stranded substrates, has also been purified. We have characterized both enzymes and have shown they form a specific and stable complex with each other. Using UV-irradiated double-stranded substrates as a model for DNA damage, we could not detect any repair synthesis nor release of thymine dimers. UV-irradiated DNA inhibited the polymerase activity in proportion to UV dose. However, treatment of the irradiated DNA with the UV-correndonuclease from Micrococcus luteus, an enzyme which nicks at the 5' side of pyrimidine dimers, stimulates the polymerizing activity several-fold. We are currently characterizing this reaction and developing assays to search for a mammalian correndonuclease and/or other factors which may interact with the polymerase·DNase V complex to stimulate repair synthesis on UV or carcinogen damaged DNA. (This work was supported by NIH grant CA17723 and American Cancer Society grant NP277.)

478 STUDIES OF THE MECHANISM OF O^{6}-METHYLGUANINE REPAIR IN DNA OF E. COLI AND RAT LIVER, Robert S. Foote, Bimal C. Pal, and Sankar Mitra, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830

We have used a synthetic DNA polymer containing O^{6}-methyl[8-^{3}H]guanine to investigate the mechanism of O^{6}-methyl-G repair in a cell-free system. Using this substrate, the adaptive response of E. coli to alkylating agents was shown to involve in situ demethylation of O^{6}-methylguanine. Incubation with an extract of E. coli which had been grown in the presence of N-methyl-N'-nitro-N-nitrosoguanidine resulted in the formation of ^{3}H-labeled guanine in the substrate. Studies of the rate and extent of demethylation support an earlier proposal that each molecule of the induced repair agent can repair only one O^{6}-methylguanine residue. Similar DNA substrates containing either ring-labeled or methyl-labeled O^{6}-methylguanine are being used to investigate the pathway of repair of this lesion in rat liver. (Research sponsored by the Office of Health and Environmental Research, U. S. Department of Energy, under contract W-7405-eng-26 with the Union Carbide Corporation.)

479 DOSE EFFECTS IN 7,12-DIMETHYLBENZ[a]ANTHRACENE-DNA INTERACTIONS, C.A.H. Bigger and A. Dipple, Chemical Carcinogenesis Program, Frederick Cancer Research Center, Frederick, MD 21701

In a variety of systems which permit metabolism-mediated binding of the potent hydrocarbon carcinogen, 7,12-dimethylbenz[a]anthracene to DNA, binding initially increases with the concentration of this hydrocarbon. However, while this quantitative relationship reflects a dose-dependent increase in the binding of the putative bay region diol-epoxide of 7,12-dimethylbenz[a]anthracene to DNA in intact cellular systems, this is not the case for cell homogenates. In the latter systems, binding to DNA is mediated by different reactive metabolites at different 7,12-dimethylbenz[a]anthracene concentrations. For example, at high concentrations binding catalyzed by microsomal or S-9 preparations occurs primarily through the intermediacy of 7,12-dimethylbenz[a]anthracene-5,6-oxide rather than the diol-epoxide and even though some diol-epoxide-DNA adducts are formed at lower substrate concentrations, these are always accompanied by other adducts not seen in the intact cellular systems. These findings limit the usefulness of such homogenate systems as models for *in vivo* metabolism and DNA binding.

480 REPAIR ENDONUCLEASES FROM LOWER EUKARYOTES: PURIFICATION AND PROPERTIES OF YEAST AND DROSOPHILA APURINIC ACTIVITIES, Susan S. Wallace, Paul R. Armel and Lola Margulies, Department of Microbiology, New York Medical College, Valhalla, New York 10595.

Apurinic DNA substrates can be formed *in vitro* or *in vivo* either directly by the action of chemical agents or indirectly by the removal of chemically damaged bases by DNA glycosylases. Thus apurinic endonucleases appear to be essential for the repair of chemically-induced damage in DNA. Such activities are ubiquitous and have been recently characterized in this laboratory from both *Saccharomyces cerevisiae* and *Drosophila melanogaster*. After partial purification by differential centrifugation and removal of nucleic acids by PEG, the yeast apurinic activity was resolved into three peaks upon DNA agarose affinity chromatography. The most tightly binding activity, which is unaffected by NEM, stimulated by 0.15 M NaCl, requires Mg^{++}, and inhibited by EDTA, was further purified by phosphocellulose and Sephadex G-100 chromatography. Two peaks of activity were resolved on phosphocellulose; the most tightly binding having a molecular weight between 55,000 and 60,000 daltons. After similar partial purification of the Drosophila enzyme, only one peak of activity was seen after DNA-agarose affinity chromagraphy. The latter activity is completely inhibited by 0.1M NaCl, 0.1 mMEDTA, 2mM $CaCl_2$ and 10 mM NEM. Mg^{++} slightly stimulates the activity at low concentrations and inhibits it at high concentrations. Both the yeast and Drosophila activities quantitatively convert AP sites to breaks in apurinic PM2 DNA.

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481 PARTIAL PURIFICATION AND PROPERTIES OF URACIL N-GLYCOSYLASE IN HeLa CELLS, Hans Krokan and Christian Wittwer, Institute of Medical Biology, University of Tromsø, Norway.

Uracil in DNA may arise from misincorporation of dUMP during DNA replication or from deamination of C. The latter process is potentially mutagenic unless repaired before the subsequent round of DNA replication. Uracil N-glycosylase removes uracil from DNA by excision of the base, leaving an apyrimidinic site. We have purified partially the human enzyme which has a molecular weight of 17000-18000 and a Km of about 10^{-6}, a value at least two orders of magnitude higher than that of the corresponding prokaryotic enzyme. Single-stranded DNA containing dUMP is about 2 fold better as a substrate than the corresponding double-stranded DNA. The enzyme is stimulated 2-3 fold by about 60 mM of monovalent salt and has no requirement for divalent cations. It is strongly inhibited by $HgCl_2$, $ZnCl_2$ and $CoCl_2$. Uracil and two analogs, 6-aminouracil and 5-azauracil, all inhibit uracil-N-glycosylase, but several other analogs have little or no effect.

482 PURIFICATION OF A 3-METHYLADENINE-DNA GLYCOSYLASE FROM HUMAN PLACENTA, Patricia E. Gallagher and Thomas P. Brent, St. Jude Children's Res. Hosp., Memphis, TN 38101

3-Methyladenine (3-MeA) is a major product in DNA treated with carcinogenic alkylating agents such as methyl methanesulfonate (MeMS). DNA glycosylases that specifically cleave 3-MeA from DNA leaving apurinic (AP) sites have been isolated from both bacteria and mammalian cells. It is presumed that these enzymes initiate the repair of this DNA lesion *in vivo*. Here we describe the presence of 3-methyladenine-DNA glycosylase in various human tissues including placenta, liver and spleen. The placental enzyme has been purified greater than 1000-fold and characterized. The routine assay employed MeMS-treated PM2 viral DNA as the substrate and a filter-binding assay to determine glycosylase produced AP-sites. The enzyme is relatively stable retaining half its activity for several days at 24°C, for about 3 hrs at 37°C and for 45 min at 45°C. No loss of activity was observed after storage for more than three months at -196°C. The glycosylase does not require monovalent or divalent ions being active in 5 mM EDTA or 5 mM EGTA. However, addition of NaCl or KCl (20-50 mM) or $MgSO_4$ (0.1-0.5 mM) results in significant stimulation of activity while higher concentrations of these salts produce inhibition. It has a broad pH optimum between pH 7.5-8.5 and displays similar activities in TRIS-HCl and HEPES-KOH buffers. The apparent molecular weight determined by Sephacryl-200 gel filtration is about 25000 daltons. The enzyme showed no detectable activity for intact DNA. Release of 3-methyladenine from ^{14}C-MeMS treated calf thymus DNA was confirmed by thin layer and Sephadex G-10 chromatography. Release of other methylated bases, including 7-methylguanine, was not detected. Additional physical and chemical properties of the enzyme will be presented. This work is supported by a grant CA-14799 from the N.I.H. and by ALSAC.

483 MECHANISMS OF INTERACTION OF BENZO(A)PYRENE DIOL EPOXIDE AND RELATED COMPOUNDS WITH DNA. CONFORMATION OF ADDUCTS. Nicholas E. Geacintov, Victor Ibanez, Hiroko Yoshida, Hanina Hibshoosh and Ronald G. Harvey, Chemistry Department,New York University,New York,N.Y. 10003.

Several different spectroscoptic techniques were utilized to investigate the mechanisms of interaction and the structure of non-covalent and covalent adducts which are formed when anti-benzo(a)pyrene-7,8-diol-9,10-epoxide (BaPDE) is reacted with DNA in aqueous solution. Stopped-flow absorption kinetic methods indicate that BaPDE forms a non-covalent intercalation complex with DNA within 5 ms or less. The absorption maximum of intercalated BaPDE is at 353 nm, shifted by 10 nm from the solution value. At pH 7.0, 25°C, 90-95% of the BaPDE is hydrolyzed to its tetraol (BPT) within 25 s, while the remaining 5-10% binds covalently to DNA. When the temperature is lowered to 0°C, this marked DNA-induced catalysis of BaPDE to BPT is inhibited, and complete hydrolysis occurs within 7-8 minutes while $\sim$30% of the BaPDE is now bound covalently to DNA. The covalent adducts display only a 2 nm red shift upon reacting with DNA (maximum at 345 nm); electric linear dichroism and fluorescence methods indicate that the conformation of the covalent adduct of BaPDE is definitely not of the intercalation-type, and that the pyrene nucleus is located on the exterior of the DNA molecule. Covalent DNA adducts formed by reacting 9,10-epoxy-7,8,9,10-tetrahydro BaP (9,10 BaPE) and the analogous 7,8 BaPE isomer display intercalation-like electric linear dichroism spectra and absorption maxima at 353 nm. Fluorescence techniques, however, indicate that these are not classical intercalation complexes and that their microstructure is different from those obtained with non-covalent aromatic dye or BPT-DNA complexes.

Carcinogen Damage to Chromatin

484 ABERRANT PHOSPHORYLATION OF NUCLEAR PROTEINS AT EARLY STAGES OF COLON CARCINOGENESIS INDUCED BY 1,2-DIMETHYLHYDRAZINE. V.G.Allfrey, L.C.Boffa, and R.Gruss, Rockefeller University, New York, NY 10021.

On the premise that phosphorylation of nuclear proteins provides a general mechanism for modulating their interactions with DNA, and may control the assembly of regulatory protein complexes, we have begun a search for aberrant phosphorylations of nuclear proteins at early stages in the induction of colonic tumors by 1,2-dimethylhydrazine (DMH). DMH was injected at weekly intervals, and 6 days after the previous DMH-injection, nuclei were isolated from the colonic epithelium following a 3-hour 'pulse' with ^{32}P-orthophosphate. Control animals, not receiving DMH, were injected with ^{32}P-orthophosphate in the same way. The nuclear proteins were then extracted and separated by high-resolution, two-dimensional gel electrophoresis. The positions of the radioactive phosphoproteins were determined by autoradiography and the autoradiograms were analyzed by computer-assisted raster-scanning microdensitometry. The results show a remarkable increase in the phosphorylation of a particular nuclear protein [MW 55,000; pI 6.4] in the DMH-treated animals. Quantitation of the radioactive protein peak at successive stages in DMH-treatment showed a maximum at 5 weeks, which is before any morphological signs of malignancy appear. Thus, a specific aberration of nuclear protein phosphorylation is an early event in chemically induced carcinogenesis of the colon.

485 ACTIVATION OF POLY (ADENOSINE DIPHOSPHATE RIBOSE) POLYMERASE WITH UV IRRADIATED AND UV ENDONUCLEASE TREATED SV 40 MINICHROMOSOME, Jonathan J. Cohen and Nathan A. Berger, Washington University School of Medicine and Jewish Hospital of St. Louis, St. Louis, Missouri, 63108

The SV 40 minichromosome was UV irradiated and used to probe the nature of the lesion responsible for UV stimulation of poly (ADPR) polymerase. SV 40 minichromosomes were prepared from purified SV 40 virus and were shown by gel electrophoresis to contain histone H2A, H2B, H3, and H4, but not histone H1. Agarose gel electrophoresis showed that the SV 40 DNA of these minichromosomes existed in two forms: form I DNA, or supercoiled, and form II DNA, or nicked circular. When minichromosomes were UV irradiated with doses between 50 and 1000 J/m^2, there was no change in the distribution of DNA on agarose gels indicating no breakes had occured in the DNA. This UV irradiated DNA did not stimulate the activity of purified poly (ADPR) polymerase. When the UV irradiated minichromosomes were treated with *M. luteus* UV endonuclease, form I DNA was completely converted to form II DNA by the 500 and 1000 J/m^2 doses. After the minichromosomes were UV irradiated and then treated with the UV endonuclease they caused a marked stimulation of poly (ADPR) polymerase. This stimulation occured inthe absence of histone H1, but was completely suppressed when histone H1 was added to the poly (ADPR) polymerase assay. These studies demonstrate in a purified *in vitro* system that damage caused by UV irradiation alone is not sufficient to stimulate poly (ADPR) polymerase activity. Only when DNA is incised at the site of UV damage by UV endonuclease is there stimulation of poly (ADPR) polymerase, and then only in the absence of histone H1.

486 REACTIVE INTERMEDIATES OF 2-ACETYLAMINOFLUORENE METABOLISM IN VITRO COVALENTLY LABEL SPECIFIC RAT LIVER MECROSOMAL PROTEINS, Tony K. Bradshaw, Robert B. Freedman and Mustach A. Kaderbahai, Shell Toxicology Laboratory, Shell Research Centre, Sittingbourne Kent, United Kingdom, ME9 8AG.

Incubation of (^{14}C)-2-acetylaminoflurene *in vitro* with rat liver microsomes, leads to covalent binding of label to microsomal proteins. The binding is NADPH-dependent, increases linearly with time and is inhibited by SKF 525A and 7,8-benzoflavone. Binding is increased more than 8-fold in microsomes from 3-methylchloranthrene-induced rats, but less than 2-fold in those from phenobarbital-induced rats. In the presence of cytosolic proteins there is slight enhancement of the labelling of microsomes and some labelling of the cytosolic proteins. SDS-PAGE and 2-dimensional gel electrophoresis indicate that covalent labelling by 2-acetylaminofluorene derivatives is concentrated in specific proteins. The major proteins labelled in microsomes from control and 3-methylcholanthrene-induced rats correspond in molecular weight to cytochrome P-450, cytochrome b_5 and their reductases. Different patterns of protein labelling are observed in microsomes from rats pretreated with phenobarbital or 2-acetylaminoflurorene. The specificity of labelling suggests that reactive intermediates may interact immediately with proteins close to the site(s) at which they are generated.

487 AFLATOXIN B_1 INHIBITION OF RAT LIVER NUCLEOLAR RNA SYNTHESIS, Fu-Li Yu, Rockford School of Medicine, Rockford, IL 61101

In an earlier report, it has been shown that aflatoxin B_1 treatment strongly inhibits rat liver nucleolar RNA synthesis (J. Biol. Chem. 252, 3245, 1977). This is an attempt to elucidate the mechanism of this inhibition. Two hours after aflatoxin B_1 injection (0.3 mg/100 g body weight), rat liver nucleolar RNA synthesis, *in vitro*, was inhibited 90%, on the average. This inhibition could result from a) inhibited RNA polymerase I activity *per se*. b) impaired nucleolar DNA template, or c) impaired nucleolar chromatin. Earlier studies found that the total RNA polymerase I activity was not affected by aflatoxin B_1 treatment. In the present work the nucleolar DNAs from both control and aflatoxin B_1 treated groups were isolated and compared for template efficiencies in directing RNA synthesis with solubilized RNA polymerase I from the control group. No difference was found. However, when nucleolar chromatin function was analyzed, it was found aflatoxin B_1 treatment resulted in a dramatic reduction in the RNA chain length to only 13% of the control size. The chain number, which is a measure of the number of engaged enzymes transcribing the nucleolar chromatin, was only slightly reduced (32%). Furthermore, since it was found that aflatoxin B_1 treatment did not increase RNase activity in the treated nucleoli, the dramatic decrease in RNA chain length is therefore believed to be a result of aflatoxin B_1 inhibition of elongation of RNA synthesis. DNase I digestion of the nucleolar chromatin suggests aflatoxin B_1 treatment may have altered the conformation of the transcriptionally active regions of the nucleolar chromatin.

488 INHIBITION OF EXCISION REPAIR OF UV-DAMAGE BY METHYL METHANESULFONATE, S.D. Park, K.H. Choi and J.E. Cleaver, Dept. of Zoology, Seoul Nat'l U. Seoul 151, Korea and Lab. of Radiobiology, UC, San Francisco, CA 94143

The rate of unscheduled DNA synthesis in normal human fibroblasts HF_1 exposed to MMS and UV-light was much less than the sum of those treated separately. Excision of pyrimidine dimers produced by UV-light was inhibited by exposure to MMS prior to UV-irradiation. These results indicate that MMS inhibits UV-induced excision repair, but repair of MMS damage was not inhibited by UV-light. Because the pathways for excision of pyrimidine dimers and alkylation damage have previously been shown to be different, this observation implies a direct effect of alkylation on repair enzymes. We estimate that if inhibition is due to protein alkylation, the UV repair system must present an extremely large target to alkylation and may involve a complex of protein subunits in the order of one million daltons such that one or more alkylations occur per complex at the concentrations used. These results also indicate that the method of exposing cells to two DNA-damaging agents to determine whether they are repaired by common or different pathways can be quite unreliable because of other effects on the repair systems themselves. Work supported by the Ministry of Education, Korea and the Department of Energy, US.

489 ALKYLATION OF DNA IN SITU BY N-METHYL-N'NITRO-N-NITROSOGUANIDINE IS NON-RANDOM AND IS DETERMINED BY REPLICON STRUCTURE, Abraham M. Stein, Carlo Ambrosino and Gerald Murison, Florida International University, Miami, FL 33199

We have studied the replication of DNA in a minimum deviation rat hepatoma cell system treated with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). On velocity sedimentation in alkaline sucrose gradients, ^{14}C-dT prelabeled cells yield paucidisperse single strand DNA fragments approximately the size of a replicon. Newly replicating DNA, labeled by ^{3}H-dT pulse-chase, elongates rapidly to a molecular weight range exactly that of the parental DNA. Further elongation is delayed for about 30 minutes, filial DNA increasing thereafter in the absence of parental DNA strand repair and at a rate lower than that of DNA elongation in untreated cells. Under conditions of complete block of DNA elongation, overall DNA synthesis is inhibited about 30 per cent as measured by ^{3}H-dT incorporation in the acid insoluble fraction. The effect of MNNG on the molecular weight and dispersity of single strand DNA fragments is concentration dependent: at low MNNG concentrations the parental single strands have higher sedimentation constants and more dispersed sedimentation patterns than the filial single strands; as concentration increases the two sedimentation patterns become more homogeneous and coalesce. Between 0.06 and 1.0 mM concentration a plateau in MNNG effect is evident, where the molecular weights of the parental and filial DNA strands are identical, and the congruent sedimentation patterns are little affected by change in MNNG concentration. These data are interpreted to indicate periodicity and hence, nonrandomness, in the site of attack of MNNG on DNA in situ. Arguments are presented to identify the domain of chromatin DNA defined by the MNNG attack with the replicon structure of DNA. The replicating unit of DNA is proposed as a periodic element of chromatin architecture.

490 STUDIES ON THE DNA LOCATION OF BENZO(A)PYRENE MOIETIES, BEFORE AND AFTER REPAIR, IN HUMAN LUNG CELLS, Janet E. Arrand, Anne M. Tyers and Robert Williamson, St. Mary's Hospital Medical School, London W2 1PG, England.

Comparative studies using epithelial-like cells and fibroblasts derived from human fetal lung have shown distinct differences in the behaviour of the cell types with respect to the extent of benzo(a)pyrene binding to DNA and its subsequent removal by repair enzymes.

Using the DNAase I procedure of Weintraub and Groudine (Science 193 848) to preferentially digest transcriptionally-active regions of the genome, we have determined the relative amounts of benzo(a)pyrene bound to regions of varying DNAase sensitivity - namely, to transcribed regions, to regions bordering transcribed genes, and to non-transcribed regions. These studies have been performed on the two cell types both at times of maximum benzpyrene binding and after repair has been allowed to proceed.

These, and further studies on benzpyrene binding to origins of DNA replication and to highly reiterated DNA sequences will be discussed.

491 THE STEP AT WHICH $(ADP\text{-}RIBOSE)_n$ IS INVOLVED IN DNA REPAIR, Barbara W. Durkacz and Sydney Shall, Biochemistry Laboratory, University of Sussex, Brighton, Sussex BN1 9QG, U.K.

We have previously shown that a specific inhibitor (3-aminobenzamide) of (ADP-Ribosyl) transferase prevents rejoining of DNA strand breaks caused by dimethyl sulphate (DMS) in mouse lymphoblastoid (L1210) cells (B.W. Durkacz et al., Nature 283, p593 (1980)). We have assayed the capacity of L1210 cells to repair damaged DNA by following the restoration of DNA supercoiling in nucleiod preparations, in the presence or absence of 3-aminobenzamide. The results lead to the following conclusions:- (1) The incision steps of repair are not inhibited; (2) At early times following damage, the number of DNA strand breaks is much higher when cells are incubated in 3-aminobenzamide; 3-aminobenzamide itself does not cause strand breakage; (3) The effect of 3-aminobenzamide on strand rejoining at later times has also been estimated. The step at which $(ADP\text{-}Ribose)_n$ is involved in DNA repair is thus shown to be after the strand-breakage step.

492 MODIFICATION OF TARGET MACROMOLECULES IN COLONIC EPITHELIAL CELLS BY 1,2-DIMETHYLHYDRAZINE. L.C.Boffa, R.Gruss, and V.G.Allfrey, Rockefeller University,New York NY 10021.

The alkylating carcinogen, 1,2-dimethylhydrazine (DMH) , induces colonic tumors with high incidence and tissue specificity. The sites of in vivo modification of target macromolecules were studied, using[^{3}H-methyl]DMH. Carcinogen binding to subcellular fractions of colonic epithelial cells was analyzed at time intervals ranging from 10 minutes to 36 hours, with particular emphasis on the alkylation of nuclear constituents. DMH modifies, not only nucleic acids, but histones and nonhistone nuclear proteins in the target cells. Separation of the ^{3}H-methylated amino acids showed aberrant methylation patterns after exposure to ^{3}H-DMH, as compared with methylations observed with [methyl-^{3}H]methionine as a natural methyl group donor. The DMH-modified colonic histones have abnormally high methylarginine contents, while the nonhistone proteins contain methyllysine residues not normally detected. All nuclear protein classes labeled with ^{3}H-DMH contain a unique methylated compound which is highly radioactive, ninhydrin-positive, but atypical in its elution from the amino acid analyzer column. Proteins of the high-mobility-group (HMG proteins), which are known to be associated with transcriptionally-active and more accessible DNA sequences, normally contain N^G,N^G-dimethylarginine, and are also methylated by the carcinogen. After exposure to ^{3}H-DMH, the level of alkylation of the HMG proteins is 3 times greater than that of the histones or nonhistone proteins in the same cells. The result suggests that carcinogen-induced chromosomal damage is not random but may selectively affect the 'active' genes in the target cells.

493 THE EFFECT OF POLY(ADPR) POLYMERASE INHIBITORS ON INTRACELLULAR POLY(ADP-RIBOSE) LEVELS AND ON DNA REPAIR IN NORMAL HUMAN LYMPHOCYTES, James L. Sims and Nathan A. Berger, Washington Univ School of Medicine at Jewish Hospital, St. Louis, MO 63110.

Poly(ADP-Ribose) is a unique biopolymer synthesized from NAD by a chromatin bound enzyme. While its exact function is unknown, increasing evidence suggests that poly(ADP-Ribose) has a role in the repair of DNA damage. It has been proposed that it may modify chromosomal proteins to cause a transient alteration in chromosome structure, thus facilitating the DNA repair process. Recently a highly sensitive and selective chemical assay has been developed which makes it possible to measure intracellular levels of poly(ADP-Ribose) (Sims et al., Anal Biochem 106:296-306, 1980). We have used this technique to examine the changes that occur in poly(ADP-Ribose) synthesis when normal human lymphocytes are treated with DNA damaging agents such as N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). MNNG treatment induces a marked increase in activity of poly(ADPR) polymerase and an associated decrease in cellular NAD levels. Under these conditions the intracellular poly(ADP-Ribose) levels increased by at least 50 to 100-fold and this increase was apparent within 10 min of treating the cells with the DNA damaging agent. Thus, the rapid synthesis of poly(ADP-Ribose) may provide the means of drastically altering chromatin structure within a short interval following induction of DNA damage. To our knowledge this is the first report of an increase in intracellular poly(ADP-Ribose) levels occurring in normal human cells following induction of DNA damage. Studies will be presented on the effects of specific polymerase inhibitors on the intracellular poly(ADP-Ribose) levels and on DNA repair synthesis following damage by MNNG.

494 THERMAL ENHANCEMENT OF DNA CROSSLINKING INDUCED BY RADIATION DAMAGE. Anne E. Cress, Rene C. Davis, and G. Tim Bowden, Division of Radiation Oncology, Univerity of Arizona Health Sciences Center, Tucson, AZ 85724.
We have found that the known radiation induced DNA-protein crosslinking can be significantly enhanced by a pretreatment of cells with hyperthermia. L1210 cells were treated with 43°C for 15 minutes and then irradiated with 50 Gy x-ray. Using stepwise alkaline elution and calculating the crosslink factor, heat and radiation results in a factor of 5.5 compared to 2.34 with a treatment of radiation alone. ^{14}C-leucine labeling studies suggest that under these enhancement conditions, the DNA protein complexes can be recovered and analyzed. We have resolved the DNA protein complexes more directly by CsCl density gradient analysis. There is a bouyant density shift between the DNA treated with and without proteinase K, isolated from cells with a crosslink factor of 5.5. The density difference is 0.01 g/cc corresponding to an approximate weight difference of 2.0%. These data suggest that the DNA protein crosslinking is directly detectable, is probably covalent (surviving 5M CsCl and 1% sarkosyl) and is recoverable (indicated by a large weight difference). The significance of these tenaciously bound protein(s) is that they could be involved in DNA transcription, replication or repair -- which are processes known to be affected by radiation. Further, heat and radiation treatments are known to enhance chromosomal aberrations and recent evidence suggests the combined treatments alter the frequency of mutagenesis and malignant transformation.

495 REPAIR OF NUCLEOSOMAL DNA IN VITRO BY A 3-METHYLADENINE DNA GLYCOSYLASE, J.A. Price, E. Heller and D.A. Goldthwait, Case Western Reserve University, Cleveland, OH 44106
Nucleosomes have been prepared from chicken erythrocytes and characterized by sedimentation in sucrose gradients and in the analytical ultracentrifuge, by numbers of base pairs, and by protein patterns. When equimolar amounts of free DNA and DNA in nucleosomes were alkylated with [^{3}H] methylnitrosourea, there was only 56% of the counts incorporated into nucleosomes compared to the free DNA. In nucleosomes, the ratio of alkylation of histone to DNA was 0.09. There was no evidence for preferential shielding of sites of alkylation in either the major or minor DNA groove as judged by ratios of 3-methyladenine to 7-methylguanine to O-6-methylguanine in nucleosomal vs. free DNA. Alkylated nucleosomes were incubated with purified 3-methyladenine DNA glycosylase I of E. coli and release of 3-methyladenine was measured over a two hour period. With three separate batches of nucleosomes incubated at 37° the release reached plateau values varying from 23 to 48% of the total 3-methyladenine with an average of 33%. At 25°, the plateau was 15%. Under all these conditions there was no significant conversion of 11S to 5S material. In preliminary experiments with subsaturating levels of ethidium bromide, there was enhancement of enzymatic release. In in vivo experiments with murine lymphoma cells, 80% of the 3-methyladenine was released in 5.5 hours. The in vitro results show that more than half of the 3-mtheyladenine residues on nucleosomal DNA is not available to the specific enzymatic probe, and they suggest that mechanisms exist which alter the binding of DNA to histones in the repair process.

496 BINDING OF BENZO(a)PYRENE DIOL EPOXIDE TO MOUSE EPIDERMAL RNA, DNA AND PROTEIN: DOES SELECTIVE BINDING OF SYN AND ANTI DIASTEREOMERS OCCUR IN EPIDERMAL PROTEINS? Jill C. Pelling and Thomas J. Slaga, Oak Ridge National Laboratory, Oak Ridge, TN 37830.
The binding of benzo(a)pyrene 7,8-diol-9,10-epoxide, (BPDE), the ultimate carcinogenic metabolite of benzo(a)pyrene, was investigated in mouse epidermal cells. In vitro studies using cultured mouse epidermal cells exposed to the diastereomeric forms of BPDE indicated that a differential binding occurred in RNA, DNA, and protein. Exposure of cultured epidermal cells to the racemic anti diastereomer of BPDE resulted in nearly twice as much carcinogen being bound to cell DNA as to total cellular protein per microgram. In contrast, syn BPDE bound in higher proportions to cell proteins, with less diol epoxide binding to DNA and RNA. To determine if either diastereomer exhibited selective binding to specific cell proteins, proteins from cultured epidermal cells exposed to anti or syn BPDE were purified and subjected to electrophoresis on polyacrylamide gels. Preliminary results suggest that anti BPDE may be bound preferentially to histone H3, whereas syn BPDE is bound selectively to high molecular weight cell proteins and possibly to histone H1. The binding of BPDE to epidermal macromolecules is also being studied in vivo after topical application of the diol epoxide to adult mouse skin. Initial experiments suggest that anti BPDE binds in equal proportions to total cell DNA and protein, with 7 to 10 times less BPDE bound to RNA. Fluorographic analysis is underway to determine whether selective binding of the BPDE diastereomers occurs in epidermal proteins in vivo.

497 VERY LYSINE-RICH HISTONES ARE NOT TARGETS FOR BENZO(A)PYRENE BINDING IN INTACT CELLS, Michael C. MacLeod, Arend Kootstra, Betty K. Mansfield, Thomas J. Slaga and James K. Selkirk, Biology Division, Oak Ridge National Laboratory and The University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences, Oak Ridge, TN 37830.

Confluent tertiary cultures of hamster embryo cells (HECs) metabolize benzo(a)pyrene [B(a)P] to derivatives which bind covalently to nuclear macromolecules, particularly nuclear proteins. In previous studies (MacLeod, et al., Proc. Natl. Acad. Sci. USA, in press) we demonstrated selectivity of binding to different proteins. In particular, a protein(s) with electrophoretic mobility similar to the very lysine-rich histones was highly labeled in incubations with tritium labeled B(a)P or B(a)P-9,10-dihydrodiol but not in incubations with B(a)P-7,8-dihydrodiol or B(a)P-7,8,9,10-tetrahydro-7,8-diol-9,10-oxide. In order to determine whether this binding was to one or more of the very lysine rich histones we have performed a number of experiments which partially separate the very lysine-rich histones from other nuclear proteins. Electrophoretic analyses demonstrate that the highly-labelled protein of apparent M_r = 30,000 (protein BP30) does not copurify with the very lysine-rich histones, but can in fact be extracted from intact nuclei under conditions which do not extract the histones. Similarities between the properties of BP30 and those of nucleoplasmin, the nucleosome assembly factor from Xenopus oocytes, are noted.

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498 REPAIR OF DNA CONTAINING FUROCOUMARIN ADDUCTS IN MAMMALIAN CHROMATIN Miriam E. Zolan, Gino A. Cortopassi, Charles A. Smith and Philip C. Hanawalt, Department of Biological Sciences, Stanford University, Stanford, CA 94305

In isolated nuclei from cultured human cells treated with 254 nm UV or chemicals whose lesions are randomly distributed with respect to nucleosome cores and linkers, newly synthesized repair patches are much more sensitive to digestion by staphylococcal nuclease than is bulk DNA. Within a few hours after the patches are synthesized this increased sensitivity is almost completely gone (1). We have confirmed this result with 254 nm UV. We have also observed it in cells containing furocoumarin adducts, expected to be formed only in linkers. This supports the hypothesis of nucleosome rearrangement following repair and suggests that even if the initial increased sensitivity of repair patches to staphylococcal nuclease is due solely to loss of proper nucleosome structure at sites of repair, during re-formation of this structure nucleosome cores are not placed precisely at their original positions on the DNA.

The rate and extent of repair replication per unit DNA in African green monkey cells irradiated with 254 nm UV was found to be the same in bulk DNA as in the 172 bp highly repeated α DNA sequences. In contrast, repair in α DNA was only about 30% of that in total DNA in cells treated with angelicin (a furocoumarin that forms only mono-adducts) followed by 360 nm light. This suggests that the chromatin structure of α DNA renders it less accessible than bulk DNA to angelicin, although it is possible that repair systems recognize these adducts less efficiently in α DNA.

(1). Lieberman MW, Smerdon MJ, Tlsty TD, Oleson FB (1979) in Environmental Carcinogenesis (Emmelot P, Kriek E, eds.) pp345-363 Elsevier, Amsterdam

499 THE ROLE OF THE NUCLEOSOMAL STRUCTURE OF CHROMATIN IN THE REPAIRABILITY OF BENZO(a)-PYRENE-DIOL-EPOXIDE DNA ADDUCTS, Motohisa KANEKO and Peter A. CERUTTI, Swiss Institute for Experimental Cancer Research, CH-1066 Epalinges s/Lausanne, Switzerland.

The implications of the nucleosomal structure of chromatin on the distribution and excisability of covalent benzo(a)pyrene-diol-epoxide I (BPDE I) adducts to DNA was investigated in confluent cultures of human skin fibroblasts. The adduct concentrations were determined in total nuclear DNA, nucleosomal DNA (165 base pairs) and nucleosomal core DNA (145 base pairs) by agarose gel electrophoresis of micrococcal nuclease digests. From these data the adduct concentrations in linker DNA and in the 20 base pair portion which distinguishes 165 base pair nucleosomal DNA from 145 base pair core DNA were calculated. At an initial adduct level in total DNA of 10 to 11 μmoles per mole DNA-P the adduct concentrations were 8 to 10 times higher in the linker DNA than in nucleosomal core DNA. Adduct removal occurred with biphasic kinetics more efficiently from linker DNA than from 165 and 145 base pair nucleosomal DNA. During the rapid phase (0 to 8 h) ∿50% adducts were removed while during the subsequent 16 h only an additional 20% were excised. After 24 h incubation 30% of the adducts persisted in the DNA, approximately half of which were located in core DNA. No major differences were determined in the adduct spectrum of total DNA at 0 and 24 h post-treatment incubation. It is concluded that the nucleosomal structure of chromatin imposes major constraints on the distribution and repairability of BPDE I DNA-adducts. (Work supported by the Swiss NF and the ASFC)

500 THE EFFECT OF HEAT SHOCK ON POLY(ADP-RIBOSE) SYNTHETASE AND ON REPAIR OF X-RAY INDUCED DNA STRAND BREAKS, Nancy L. Nolan and William R. Kidwell, National Institutes of Health, Bethesda, Md. 20205

Several laboratories have proposed that the chromosomal enzyme, poly(ADP-ribose) synthetase, is important for DNA repair. In evaluating this possibility, we have found that the synthetase can be completely inactivated by a mild heat shock of Drosophila melanogaster tissue culture cells with negligible effects on cell viability and total RNA polymerase activity. During the one hour period following heat shock (5 min at 37°C), when synthetase activity is less than 10% of non heat shocked controls, the cells are unable to rejoin X-ray induced DNA chain breaks as shown by alkaline sucrose gradient analysis. Following the one hour lag, poly(ADP-ribose) synthetase activity is exponentially regenerated to 55% of pre-heat shock control levels by 2½ hours post-heat shock at 25°C. The capacity for rejoining DNA chain breaks returns to non heat shocked control levels with similar kinetics of recovery. Although there is a positive correlation between the regeneration of synthetase activity and DNA rejoining activity during heat shock recovery, conclusions regarding the synthetase involvement in repair of X-ray induced DNA lesions are reserved because, 3-aminobenzamide, a potent inhibitor of poly(ADP-ribose) synthetase in nuclei, barely effected the DNA chain rejoining activity. Heat shock may be a very useful way to evaluate the possible role of poly(ADP-ribose) synthetase in DNA repair and/or other processes. The unusually high heat sensitivity of the synthetase may also be pertinent in the synergistic effect noted in the therapeutic combination of hyperthermia and radiation or chemotherapy.

501 EFFECT OF HISTONE ACETYLATION ON THE FORMATION AND REMOVAL OF COVALENT BENZO(a)PYRENE CHROMATIN ADDUCTS, Arend Kootstra and Peter A. Cerutti, Swiss Institute for Experimental Cancer Research, CH-1066 Epalinges s/Lausanne, Switzerland.

The formation and removal of adducts induced by ^{3}H-benzo(a)pyrene-diol-epoxide I in nuclear DNA and histones was compared between human fibroblasts, which had been pre-treated with butyrate and untreated controls. Butyrate treatment causes hyperacetylation of histones H4 and H3. The following results were obtained : (1) Histone-hyperacetylation did not affect the initial level of carcinogen binding to DNA, nor did it affect the extent and specificity of the carcinogen binding to histones H3 and H2A. (2) Since the unacetylated, mono- and di-acetylated forms of histone H3 all reacted to a similar extent with the carcinogen we suggest that the ε-amino groups of the lysine residues of H3, which participate in acetylation, are not accessible to the carcinogen. (3) Histone hyperacetylation had an accelerating effect on the removal of DNA adducts. In the butyrate pretreated cultures 51% of the adducts had been removed within 24 h, while only 28% had disappeared from the DNA of untreated controls. (4) The level of carcinogen adducts to histones remained constant during 24 h post-treatment incubation. Our data suggest that histone acetylation may modulate the repairability of DNA lesions possibly by inducing conformational changes in chromatin.

This work has been supported by the Swiss National Science Foundation and the Swiss Association of Cigarette Manufacturers.

502 CARCINOGEN-DNA INTERACTION AND REPAIR ARE MODULATED BY CHROMATIN CONFORMATION, Lewis V. Rodriguez[1], Gerard Metzger[2], Abby L. Maizel and Frederick F. Becker[1], Section of Experimental Pathology[1], M. D. Anderson Hospital and Tumor Institute, Houston, TX 77030 and Department of Biology[2], University of Texas at Dallas, Richardson, TX 75080

The in vivo binding of the carcinogens N-2-acetylaminofluorene (AAF) and N-hydroxy-2-AAF (N-OH-AAF) to nuclear DNA of rat liver and the repair of damage that resulted, were determined in chromatins of varying conformational states. In an attempt to correlate in vivo carcinogen binding with the conformational state, isolated native chromatin and chromatin subpopulations were probed in vitro with the direct-acting analogue, N-acetoxy-N-2-AAF (N-AcO-AAF). To determine chromatin structure, the various preparations were analyzed for their circular dichroic spectra, ethidium bromide intercalation and thermal denaturation. Further polynucleotide ligase was utilized as a biological probe to ascertain the contribution of chromatin conformation to the detection and repair of DNA damage. The results of these studies revealed an inverse relationship between chromatin conformational restraint, carcinogen-binding and repair. Thus, DNA from chromatins having physical characteristics which indicated a more condensed conformation bound less carcinogen than those exhibiting a less condensed conformation. This was consistent both in vivo and in vitro. In addition, when examined in vivo, the less condensed forms underwent repair as reflected in removal of carcinogen adducts more rapidly. (Supported by NIH grants CA 20131 and CA 21927 and Grant AT-480 from the Robert A. Welch Foundation).

503 A MODEL OF EXCISION REPAIR IN MAMMALIAN CELLS, Klaus Erixon, University of Stockholm S-106 91 Stockholm, Sweden.

The number of single-strand breaks (SSB) present in human fibroblasts after UV-irradiation or treatment with N-acetoxy-2-acetylaminofluorene (3 AF) has been followed as a function of time and dose, both in the absence and presence of 1-ß-D-arabinofuranosylcytosine (ara C), a potent inhibitor of DNA synthesis. The "DNA unwinding technique" was used (1). In the presence of ara C, SSB accumulate in a dose-dependent fashion, but with a definite limit. UV-induced repair incorporation in the presence of ara C, as a function of time and dose, also exhibits a definite limit, suggesting that the presence of ara C only allows a certain number of damaged sites to be recognized and incised. The number of SSB obtained by combinations of UV and 3 AF are additive in the absence of ara C but show the same limit, about 5 SSB/10^8 dalton, in the presence of ara C.

Novobiocin, an inhibitor of gyrase in prokaryotes, will also inhibit UV-induced incision, as well as activity that increases negative supercoiling in mammalian DNA. A model is proposed, where binding and incision of an endonuclease is dependent on a supercoiled loop of DNA, of the same size as a replicon. As the first incision is made at a lesion, the loop will relax, and further incision is not possible until repair has restored a closed loop, whereupon the loop may be returned to its supercoiled form. The kinetic properties and predictions of this repair cycle will be discussed.

(1) Ahnström, G., and K. Erixon (1980). The measurement of strand breaks by DNA unwinding in alkali and hydroxyapatite chromatography. In: DNA Repair: A Laboratory Manual of Research Procedures. E.C. Friedberg and P.C. Hanawalt, eds. Marcel Dekker, New York (in press).

504 CARCINOGEN-INDUCED ALTERATIONS IN RESTRICTION OF NUCLEAR RNA: A RELATIONSHIP WITH RNA TRANSPORT AND NUCLEAR-ENVELOPE NUCLEOSIDE TRIPHOSPHATASE ACTIVITY. Gary A. Clawson and Edward A. Smuckler, Dept. Pathology, University of California, San Francisco, CA

Altered transport (restriction) of nuclear RNA sequences is a general response of liver cells to hepatocarcinogen treatment. This process of RNA transport is energy-requiring; energy is derived from hydrolysis of high-energy phosphate bonds by a nucleoside triphosphatase (NTPase) associated with the nuclear envelope. Following thioacetamide intoxication, large increases in nuclear envelope NTPase occur which relate to increased RNA transport, as demonstrated in vitro: Additional studies demonstrated a temporal relationship between increases in this NTPase and RNA transport assessed in vivo. Extending these studies, we have found that the carcinogens dimethylnitrosamine, aflatoxin B1, and 3'-methyl-4-dimethylaminoazobenzene all induce highly significant changes in nuclear envelope NTPase (with concommitant changes in RNA transport). We propose that modulation of nuclear envelope NTPase (and hence RNA transport) by carcinogens is somehow related to altered restriction of nuclear RNA, and that alterations in these two phenomena may be integrally linked to initiation of carcinogenesis.

505 THE EFFECT OF CARCINOGENS ON POLYNUCLEOSOME CONDENSATION AND DNA REPAIR INDUCED BY POLY ADP-RIBOSYLATION, Mark Smulson, Tauseef Butt, Androulla Lambrianidou, May Wong and Najma Malik, Department of Biochemistry, Georgetown University School of Medicine, Washington, D.C. 20007.

Poly (ADP-rib) polymerase is bound to the internucleosomal region of chromatin. A variety of carcinogens and DNA strand breakers cause activation of this enzymatic activity. Because of these effects we have recently attempted to correlate the structural domains of chromatin and the nuclear proteins which are targets for both carcinogens and poly ADP-ribosylation. Due to the higher-ordered folding of chromatin, core nucleosomal histones, histone H1, and the polymerase itself all undergo extensive poly (ADP-rib). New information, indicates that, in vitro, poly (ADP-rib) causes extensive polynucleosome crosslinking or condensation, in a NAD concentration-dependent fashion. The data suggests that long (>90) poly (ADP-rib) chains are generated due to carcinogens which cause extensive nuclear protein crosslinking, and that this, in turn, leads to polynucleosome aggregation. Data will be described to indicate that dimerization of histone H1 by poly (ADP-rib) appears to be prominant in this condensation reaction. If poly (ADP-rib) is an important reaction in carcinogen-induced DNA repair, a pivotal point to ascertain is whether nucleosomes within chromatin, proximal to DNA damage/repair are extensively ADP-ribosylated compared to regions of chromatin, distal to the damage. Accordingly, data will be described, using as a probe both the above noted polynucleosome aggregation, as well as monoclonal antibody to poly (ADP-rib) polymerase to enrich from bulk chromatin polynucleosomes undergoing both poly (ADP-rib), as well as DNA repair reactions. Supported by Grants CA 25344 and CA 13195 from NIH.

506 POLY(ADP-RIBOSE) SYNTHESIS FOLLOWING DNA DAMAGE IN CELLS HETEROZYGOUS OR HOMOZYGOUS FOR THE XERODERMA PIGMENTOSUM GENOTYPE, Myron K. Jacobson and Lynette Salter McCurry, Departments of Chemistry and Biochemistry, North Texas State University/Texas College of Osteopathic Medicine, Denton, Texas 76203.
Treatment of normal human cells with DNA damaging agents such as UV light or N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) stimulates the conversion of NAD to the chromosomal polymer poly(ADP-ribose) which in turn results in a rapid depletion of the cellular NAD pool. (Juarez-Salinas et al. Nature 282, 740 (1979), Jacobson et al. Can. Res. 40, 1797 (1980)). We have studied the effect of UV light or MNNG on the NAD pools of 7 cell lines of human fibroblasts either homozygous or heterozygous for the xeroderma pigmentosum (XP) genotype. Following UV treatment the NAD content of XP cells was unchanged (complementation groups A and D) or only slightly reduced (complementation group C). UV treatment of XP cells of the variant genotype caused a large reduction in the size of the NAD pool. Cell lines derived from asymptomatic, parental heterozygotes of XP complementation groups A and D showed a rate of conversion of NAD to poly(ADP-ribose) following UV treatment that was approximately one-half that of the control cell line. All of the cell lines had a greatly reduced content of NAD following MNNG treatment. The results demonstrate a close relationship between the conversion of NAD to poly(ADP-ribose) and DNA excision repair in human cells. (Supported in part by Grant CA23994 from the National Institutes of Health, Grant B-633 from The Robert A. Welch Foundation, and by the NTSU Faculty Research Fund.)

DNA Repair and DNA Replication

507 INHIBITION OF REPLICON INITIATION AND DNA CHAIN ELONGATION IN ULTRAVIOLET-IRRADIATED HUMAN FIBROBLASTS, *William K. Kaufmann and James E. Cleaver*, Laboratory of Radiobiology, University of California, San Francisco, CA. 94143

The inhibition of DNA replication in UV-irradiated human diploid fibroblasts was evaluated in terms of radiation effects on replicon initiation rate and DNA chain elongation. Conditions for steady-state labeling of nascent DNA molecules were established, and the distribution of sizes of nascent DNA and the fraction of growing points in operation quantified at various times after irradiation. After fluences less than 1 J/m^2, initiation of replicons was inhibited in normal human fibroblasts, in xeroderma pigmentosum (XP) fibroblasts with moderate to severe reparative deficiencies, and in XP fibroblasts belonging to the variant class. Inhibition of replicon initiation by UV, therefore, was not strictly related to the operation of the nucleotidyl excision repair pathway. XP fibroblasts with reparative deficiencies were more sensitive to UV-induced inhibition of DNA chain elongation than were normal fibroblasts presumably due to their inability to remove pyrimidine dimers ahead of DNA growing points. Although XP variants are proficient in dimer removal they displayed even greater sensitivity to inhibition of chain elongation than the deficient XP fibroblasts. This analysis suggests that DNA growing points are blocked in the XP variant at lesions with no apparent effect on chain growth in normal fibroblasts.

508 EVIDENCE FOR THE INVOLVEMENT OF LESIONS OTHER THAN O^{6}ALKYLGUANINE IN MAMMALIAN CELL MUTAGENESIS. M. Fox and J. Brennand, Paterson Labs, Christie Hospital, Manchester UK.
Newbold et al Nature 283 596 1980 reported that similar numbers of HGPRT$^-$ and oua^R mutants were induced/μMO6MeG/molDNA P by MNU, DMS and MMS in V79 cells and suggested that this indicated an important role for 06MeG in mutagenesis and carcinogenesis. We have tested this hypothesis in a reverse mutation assay HGPRT$^-$ → HGPRT$^+$ in V79 cells and present evidence for the role of lesions other than 06MeG. An HGPRT$^-$ V79 cell line which reverts spontaneously to HGPRT$^+$ (frequency 2 x 10^{-8}) reverts readily (frequency 1 x 10^{-5}) on exposure to EMS and MNU both of which produce significant levels of 0-atom alkylation. At equitoxic doses DMS and MMS were 40-60 less effective. The levels of reaction of ^{14}C EMS, MNU and DMS with DNA purines were measured, and previously reported 06/N7 ratios and the lack of ability of V79 cells to remove 06MeG confirmed. V79 cells were also unable to remove 06EtG. No quantitative correlation between levels of 06Alkyl G and numbers of revertants induced was demonstrated (Table 1).
Table 1 Relationship between reverse mutation frequency and amount of 06-alkylguanine induced by different alkylating agents.

Agent	Dose	Surviving Fraction	No$_6$ revertants/ 10^6 surviving cells	μMol O^{6}alkyl G/mol DNA P	Revertants/μMol O^{6}alkylG
DMS	0.8mM	0.73	0.27(1)	0.79	0.34
MNU	0.5mM	0.76	24.0(150)	9.5	2.5
EMS	8.1mM	0.98	16.0(134)	3.1	5.2

This suggests an important role for lesions other than O6AlkylG in mammalian cell mutagenesis.

509 ROLE OF ERROR-PRONE DNA REPLICATION IN MAMMALIAN MUTAGENESIS/CARCINOGENIC INITIATION, J.E. Trosko, S.T. Warren, M. Wade, P. Liu, and C.C. Chang, Michigan State University, East Lansing, MI 48824

Carcinogenesis appears, in many systems, to be composed of initiation and promotion phases. Mutagenesis seems to be a plausible explanation for the initiation phase. DNA replication is a multistep process involving the concerted regulation of various enzymes, all of which play an interdependent role in maintaining the fidelity of the replicating unit. Alterations of this process, due to mutations or xenobiotic influence, can lead to an increase in the error-rate during DNA synthesis. Furthermore, DNA synthesis, whether scheduled or unscheduled, may be affected leading not only to an increased spontaneous mutation rate but also to hypermutability following DNA damage. The hypermutability of excision-repair defective xeroderma pigmentosum cells, as well as of various DNA repair deficient mutants of Chinese hamster cells, has implicated the role of unrepaired DNA damage in the template DNA in induced mutagenesis. We now have evidence from the hypermutability of (1) a UV-sensitive aphidicolin-resistant mutant (a thymidine auxotroph with an altered nucleotide pool and growth rate) and (2) Bloom syndrome fibroblasts that abnormal DNA replication of a normal DNA template could also contribute to spontaneous and induced mutagenesis or carcinogenic initiation. Our data on Bloom syndrome cells indicate that these cells have a 10-fold increase in the spontaneous mutation rate, which, in conjunction with previous data, suggests that the basic error in Bloom syndrome is in DNA replication rather than DNA repair. [Research was supported by an NCI grant (PO1-CA26803) to J.E.T. and an NIEHS grant (ES01809) to C.C.C.].

510 DIFFERENTIAL EFFECT OF HYDROXYUREA ON REPLICON JOINING AND POST-REPLICATION REPAIR IN MOUSE MELANOMA CELLS, Helene Z. Hill, Marshall University School of Medicine and the Veterans Administration Medical Center, Huntington, West Virginia 25701.

Earlier work (Hill, HZ and Setlow, RB, Cancer Research 40:1867, 1980) suggested that replicon joining (RJ) and post-replication repair (PRR) are different, as 4 mouse cell lines had the same rate of RJ and different PRR rates. The B16CL4 subclone of B16 melanoma was used for these studies as it has a high rate of PRR but performs little or no excision repair after UV. PRR was studied by treating ^{14}C-prelabelled cells with 10J/m^2 of UV followed one-half hour later by a 34 minute pulse of ^{3}H-dThd. The cells were then chased in medium containing non-radioactive dThd with and without hydroxyurea (HU). At the end of the experiment, the cells were treated with 2Krad of 50KVP x-rays, lysed and centrifuged through a 5 to 20% alkaline sucrose gradient. For RJ, the UV step was omitted. In 5 experiments, the rate of RJ averaged 0.41 and that of PRR averaged 0.27 in the absence of HU, while the RJ rate averaged 0.030 and the PRR rate averaged 0.16 in the presence of concentrations of HU that completely inhibit net DNA synthesis. When the length of the pulse for the non-UV'd cells was shortened to 20 minutes to equalize the amounts of DNA synthesized during the pulses, the rate of RJ in the presence of HU increased to 0.16 in 2 experiments. RJ and gap-closing opposite pyrimidine dimers thus appear to require different enzymatic machinery than that required for chain elongation. Furthermore, the dependence of the inhibition of RJ by HU on the length of the pulse suggests that replicons occur in clusters. After a short pulse, small replicons are poised to join, while after a long pulse, the small replicons have joined and the inter-cluster distances are too great for further joining to occur.

511 RATIONALIZATION OF THE BIOLOGICAL CONSEQUENCES OF DNA DAMAGE PRODUCED BY ANTHRAMYCIN IN REPAIR PROFICIENT AND DEFICIENT HUMAN CELL LINES. Laurence H. Hurley and Ruby L. Petrusek, College of Pharmacy, University of Kentucky, Lexington, KY 40506.

The results described in this abstract show that (1) anthramycin produces excision dependent single-strand (ssb's) and double-strand (dsb's) breaks in DNA; (2) unscheduled DNA synthesis (UDS) proceeds in repair proficient cell lines without apparent removal of ssb's; and (3) anthramycin is nonmutagenic but highly recombinogenic in yeast. These results are rationalized on the basis of the known structure of the anthramycin-DNA adduct.

Anthramycin, a potent antitumor agent, reacts with DNA in a unique manner and is covalently attached through N-2 of guanine by an inherently unstable aminal linkage but the drug does not distort DNA [Hurley and Petrusek, Nature 282, 529 (1979)]. Anthramycin induces UDS in repair proficient but not a u/v excision defective strain of XP [XP12 (SV40) RO] [Hurley, et al., J. Biol. Chem. 254, 605 (1979)]. Ssb's were monitored using hydroxylapatite chromatography, dsb's by neutral sucrose gradient analysis and viscoelastrometry measurements and UDS by the BND-cellulose chromatography method. The dsb's are rationalized by a model in which repair complexes are unable to discern to which strand the anthramycin is attached, and can approach simultaneously from both directions causing near opposite ssb's in DNA. The continued UDS in the absence of removal of ssb's is rationalized on the basis of the inherent instability of the adduct when the DNA is denatured during repair, resulting in release of unchanged drug and subsequent rebinding to DNA, ad infinitum. The genetic effects are rationalized on the basis of the absence of residual template damage when DNA is denatured (nonmutagenic) and the production of dsb's in DNA (recombinogenic effects). Supported by grant CA-17407 from NCI.

512 On the mechanism of sister chromatid exchanges (SCE) induction. Georges Renault, Alain Gentil and Ivan Chouroulinkov. IRSC-CNRS, Villejuif - France

SCE's are well correlated with the mutagenic and carcinogenic properties of DNA damaging agents. Since this correlation is not well understood, we carried out experiments with X rays in order to look at the correlation of SCE induction with the cell cycle. V79 Chinese hamster cells were labelled with BrdUrd for only 6 hours, this technique allows an "a posteriori" synchronisation, and were irradiated with 400 rad of 200 kV X rays at different times before harvesting for chromosome preparation.

Our results show that an interaction between BrdUrd and X rays occurs during the first S-phase -where the BrdUrd content per cell is not the same at the moment of each irradiation-, and that SCE induction decreases with the progression of the second S-phase.

The results show clearly that SCE occurs during the S-phase.

513 RELATIONSHIP OF SISTER CHROMATID EXCHANGES TO PYRIMIDINE DIMER AND DNA BREAK INDUCTION WITH 365 nm LIGHT, Pamela L. Derstine, Viola M. Griego and Tatsuo Matsushita, Argonne National Laboratory, Argonne, IL 60439

The assay of sister chromatid exchanges (SCEs) is increasingly being used as a short term test to assess the toxicity of chemical carcinogens. We have recently adapted this technique to a mouse myeloma cell suspension line and have used SCEs as a toxicity screen for potential adverse health impacts of coal gasification. Additional insight into the mechanism underlying SCE induction would permit a more meaningful interpretation of SCE results. Therefore, we have compared SCE induction by monochromatic 365 nm light under different irradiation conditions (oxygen presence or absence, and temperature). The effect of oxygen on survival, pyrimidine dimers, single-strand DNA breaks (SSB) and SCE induction by 365 nm light was consistent with the results in E. coli reported by Tyrrell [Photochem. and Photobiol. (1976) 23, 13]. Survival, SSBs, and SCE induction per unit fluence were oxygen-dependent while dimer induction was not. Comparing the effects of irradiation at room temperature and 0° suggested that, as in E. coli, aerobic irradiation at 365 nm resulted in disruption of repair enzymes. SCE induction was positively correlated with dimer formation at 254 nm, but not at 365 nm under aerobic, room temperature conditions. SSB formation was not correlated with SCE induction under any irradiation condition. These data are not consistent with SCE mechanisms requiring DNA repair activity, but are consistent with a mechanism involving the inhibition of DNA replication [Painter (1980) Mutation Res. 70, 337]. (This work was supported by the U. S. Department of Energy under contract No. W-31-109-ENG-38.)

514 SENSITIVITY OF EXPOENTIALLY GROWING POPULATIONS OF Escherichia coli TO PHOTOINDUCED PSORALEN DNA INTERSTRAND CROSSLINKS, A.Zaritsky[1], N.B.Grover[2], E.Ben-Hur[3], A,Margalit[2] and M.T.Hansen[4]; Ben-Gurion University, Beer-Sheva (1), Hebrew University, Jerusalem (2), Nuclear Research Center, Beer-Sheva (3), Israel and Risø National Laboratory, Roskilde, Denmark (4).

The lethal action of 4,5'-8-trimethylpsoralen and near UV on Escherichia coli K12 was shown to arise exclusively from interstrand crosslinks, cell vulnerability increasing markedly with the doubling time of the culture.

Theoretical survival curves were computed for populations with defined chromosome configurations, based on two different models. The first assumes that a cell survives if at least one copy of its genome remains undamaged, but completely fails to describe the experimental results, whether or not repair by DNA strand exchange is permitted. The second model allows for a limited period of time during which DNA repair can take place, but a single crosslink in a stretch of DNA due to be replicated within this interval constitutes a fatal lesion. This model agrees rather well with the measured data over wide ranges of crosslink concentrations and doubling times. This model predicts a repair mechanism to remove crosslinks from bacterial DNA, the efficiency of which is highly growth rate dependent.

515 INTERACTION OF BENZO(a)PYRENE WITH DNA IN A HAMSTER TRACHEAL EPITHELIAL CELL LINE, Alan Eastman and Edward Bresnick, Department of Biochemistry, The University of Vermont College of Medicine, Burlington, Vermont 05405

A cloned cell line derived from a normal hamster tracheal epithelium has been characterized with respect to its response to the carcinogen benzo(a)pyrene (BP). Early passages were used as these did not give rise to tumors when reimplanted into hamsters. These cells metabolize BP to ultimate reactive forms which alkylate DNA. Alkylation with radiotracer amounts of BP was maximum at 8 hr and remained fairly constant to 48 hr. BP-containing medium was replaced with fresh medium at 4 hr, rapid removal of adducts was seen for the first 4 hr postincubation followed by a slower repair. About 50% of the DNA-bound hydrocarbon remained after 48 hr. Cells were able to divide in the presence of these lesions, undergoing 5 doublings while only 60% of the adducts were removed. Integrity of DNA during this repair period was monitored by the alkaline elution technique. A toxic dose of BP was required to cause minimal single strand breakage which was observed from 2 - 8 hr of BP treatment but at 15 hr DNA appeared normal. Comparison was made with a nontoxic dose of methyl methanesulfonate which caused very rapid elution of DNA after only 1 hr treatment. At least 12 deoxyribonucleoside-bound BP adducts were separated by high pressure liquid chromatography. Two adducts, possibly deoxyadenosine-BP, were removed within 8 hr. Other adducts were markedly reduced by 48 hr while three exhibited little if any reduction. These differential rates of repair provide a model for studying the possible significance to neoplasia of persistent and repairable lesions. Supported by CA 23514.

516 **FACTORS AFFECTING THE TERMINATION OF IN VITRO DNA SYNTHESIS ON CARCINOGEN-REACTED TEMPLATES,** **Bernard Strauss, Peter Moore* and Samuel Rabkin, University of Chicago, Chicago, IL 60637**

Carcinogen-reacted ØX174 DNA primed with restriction fragments has been used to study the termination of in vitro DNA synthesis. Electrophoresis in agarose or non-denaturing 6% polyacrylamide gels determines elongation along the whole of the DNA; denaturing PAGE determines the exact nucleotide at which synthesis terminates. The nature of the lesion, the polymerase and the metal ion(s) present affect the site of termination. Synthesis catalyzed by E. coli polymerase I (pol I) in the presence of Mg^{2+} terminates one nucleotide before the first pyrimidine in a UV-induced dimer and one nucleotide before guanine reacted with N-acetoxy-N-2-acetylaminofluorene (AAAF). When Mn^{2+} is present as the divalent cation, synthesis usually terminates at the level of the AAF-reacted base or at the first pyrimidine in a dimer. Synthesis catalyzed by AMV reverse transcriptase terminates at the level of the AAF-reacted nucleotide but one nucleotide before a dimer. AAAF reacts more than 95% at the C-8 position of guanine in ØX174 single stranded circles and linear fragments. In order to determine the synthesis events at the junction of double stranded DNA and single stranded DNA containing an AAF lesion at the first base, we are determining the nucleotide inserted opposite the reacted guanine. Synthesis is carried out with T4 polymerase which behaves like pol I; the reacted DNA is isolated and used as a substrate with AMV reverse transcriptase. **Supported by grants from the National Institutes of Health (GM 07816, CA 19265) and the Department of Energy (EY75 S-02 2040).**

*Senior Author.

517 SPECIFIC CHANGES IN DNA REPLICATION INTERMEDIATES IN PRIMATE CELLS TREATED WITH A DIHYDRODIOL EPOXIDE DERIVATIVE OF BENZO[A]PYRENE. Tim Bowden, Vincent McGovern, and Nina Ossanna, Division of Radiation Oncology, College of Medicine, University of Arizona, Tucson, AZ 85724.

African green monkey cells (Vero) were pulse labeled with [^{3}H] thymidine at various times after treatment with (±)-7 β, 8 α dihydroxy-9 α,10 α epoxy-7,8,9,10 tetrahydrobenzo[a]pyrene (B[a]P-diol epoxide I). The size of nascent DNA after treatment showed a dose dependent decrease (1-2 hr.) and a compensatory increase (6-10 hr.) while the rate of DNA synthesis showed a greater dose dependent decrease followed by a partial recovery at the lower dose studied (0.66 μM) and no recovery at the toxic higher dose (1.66 μM). DNA replication intermediates were studied using a pH-step alkaline elution technique. Using x-irradiation we found that pH 11.0 (1.5×10^5 daltons) and pH 11.3 (13.4×10^6 daltons) eluted nascent DNA of the indicated size involved in DNA replication initiation while pH 11.5 (38×10^6 daltons) and pH 12.1 ($51\text{-}71 \times 10^6$ daltons) eluted elongated nascent DNA. Selective dose dependent increases in the relative amount of nascent DNA eluting at pH 11.0 and pH 11.3 and decreases of nascent DNA eluting at pH 11.5 and 12.1 were observed after B[a]P-diol epoxide I treatment. Recovery toward control distributions of replication intermediates was noted (6-12 hr.). The relative increases in nascent DNA associated with DNA initiation could not be accounted for by an increase in the number of initiation sites. Therefore B[a]P-diol epoxide I blocks replication in functioning replicons through inhibition in DNA elongation with no effect on DNA initiation. We have also provided evidence that this block to elongation is overcome with time.

518 EXCISION REPAIR OF AFLATOXIN B_1-DNA ADDUCTS IN HUMAN FIBROBLASTS, Steven A. LEADON and Peter A. CERUTTI,
Swiss Institute for Experimental Cancer Research, CH-1066 Epalinges s/Lausanne, Switzerland.
The removal of covalent AFB_1-adducts from DNA was studied in confluent cultures of normal human fibroblasts. Rat liver microsome activation of AFB_1 was used which resulted in the induction of 2,3-dihydro-2-(N^7-guanyl)-3-hydroxy-aflatoxin B_1 (AFB_1-N^7-Gua) to more than 90%. Removal of adducts was compared during 48 h post-treatment incubation between DNA in situ in the cell and DNA isolated from the same cells and incubated under physiological conditions in vitro. The removal of total adducts was considerably faster in situ where 55% had disappeared in 20 h relative to only 20% from free DNA. This difference in kinetics was mostly due to more efficient removal of AFB_1-N^7-Gua in the cell. The formation of the chemically more stable secondary product which gives rise to the putative 2,3-dihydro-2-(N^5-formyl-2',5',6'-triamino-4'-oxo-N^5-pyrimidyl)-3-hydroxy-AFB_1 upon acid hydrolysis was also slightly more rapid in the cell relative to free DNA. It is concluded that human fibroblasts possess the capacity to actively excise AFB_1-N^7-Gua from their DNA. (Work supported by the Swiss NF)

519 FORMATION OF MUTAGENS DURING COOKING OF PROTEIN FOODS, F.T. Hatch, J.S. Felton, S. Healy, D. Stuermer, P. Berry, and H. Timourian, Lawrence Livermore National Laboratory and L.F. Bjeldanes and M. Morris, Dept. of Nutritional Sciences, Univ. of Calif., Livermore and Berkeley, CA.
Ground beef was fried at 200°C (392°F) to a well-done, non-charred state. The extracted organic base fraction was highly mutagenic (reversion to histidine prototrophy) with S9 activation in Salmonella strains TA 1538 (6300 revertants per 100 g fresh wt of meat), TA 1537 and TA 98, which are sensitive to frameshift mutations; but showed no activity in strains sensitive to base substitution mutations. Treatment of the organic base fraction with $NaNO_2$ reduced the mutagenic activity in TA 1538 by 85%, suggesting that at least part of the mutagen is a primary or secondary amine. Neutral and acidic extracts showed no significant mutagenesis in any of the five strains. Priorities for testing other protein foods were computed from data bases of U.S. Dept. of Agriculture (1965-1966) and U.S. Dept. of HEW (Health and Nutrition Examination Survey, 1971-1975). The major sources of cooked protein intake were beef, eggs, pork, ham, bacon and fried chicken. Significant mutagen content was found in all of the above foods when cooked well-done by frying, deep-frying, or broiling as appropriate. Low or negligible mutagen content was observed under normal cooking conditions in fried shrimp, rock cod (pan-fried, baked, poached and broiled), tofu (cooked several ways), kidney and pinto beans (boiled, fried or baked). Steak contained negligible mutagen when pan-broiled, stewed, or braised; significant mutagen was present after extensive oven-broiling and roasting. Sponsored by U.S. DOE under Con. #W-7405-ENG-48 and National Institute of Environmental Health Sciences under Interagency Agreement #222Y01-ES-80038.

520 CONSEQUENCES OF REPAIR OF O^6-MEGUA ON THE DIFFERING SENSITIVITIES TO TRANSFORMATION OF SYNCHRONOUS AND ASYNCHRONOUS 10T1/2 CELLS, G.J. Smith, J.W. Grisham, and D.G. Kaufman, University of North Carolina Medical School, Chapel Hill, N.C. 27514.
When 10T1/2 cells are induced to proliferate synchronously they are sensitive to transformation by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) although logarithmically proliferating 10T1/2 cells resist transformation after comparable treatment. Since synchronously and asynchronously proliferating 10T1/2 cells treated with doses of MNNG yielding equivalent toxicity have similar quantities of O^6-meGua and N^7-meGua in their DNA, we examined whether the differing sensitivities to neoplastic transformation reflected differences in the rate of removal of these altered bases from DNA. In synchronously proliferating cells, O^6-meGua was lost from DNA at the average rate of 2.4%/hr during G_1 phase but was not removed in the S phase. The 48% reduction observed during the S phase could be accounted for precisely by dilution due to the doubling of the cellular DNA during replication. The pattern of loss of N^7-meGua during G_1 and S phases was similar. In asynchronously proliferating 10T1/2 cells, O^6-meGua was lost at the average rate of 1.4%/hr. Assuming in analogy to synchronous cells, that asynchronous cells also do not remove O^6-meGua during S phase, the non-S phase cells also removed O^6-meGua at a rate of about 2.4%/hr. During a shorter G_1 phase, asynchronous cells remove only 18% of O^6-meGua yet resist neoplastic transformation, whereas synchronous 10T1/2 cells remove 35% of O^6-meGua during G_1 phase but are sensitive to transformation. Consequently, the differences in susceptibility to transformation of asynchronously and synchronously proliferating 10T1/2 cells cannot be explained by differences in repair capacities for O^6-meGua. Supported by NIH grants CA 24144 and RCDA CA 00431 (D.G.K.).

521 IS DNA POLYMERASE α INVOLVED IN DNA REPAIR?, Elaine L. Jacobson, Dolores Juarez and D. Michael Payne, Texas Woman's University, Denton, TX 76204

Aphidicolin, an antibiotic produced by *Cephalosporium aphidicola*, has been shown by numerous workers to be a specific inhibitor of DNA polymerase α *in vitro*. It has also been demonstrated to be a potent inhibitor of DNA replication both *in vivo* and *in vitro*. Reports from two laboratories indicate that aphidicolin inhibits repair replication *in vitro* and *in vivo*, implicating an involvement of DNA polymerase α in DNA repair as well as replication (Berger, et al. Biochem. Biophys. Res. Commun. 89, 218 (1979) and Hananoka, et al. Biochem. Biophys. Res. Commun. 87, 575 (1979)). A more recent report is in direct conflict with these observations in that repair replication is reported to be unaffected by aphidicolin over a wide range of concentrations (Pedrali-Noy and Spadari, Mut. Res. 70, 389 (1980). These authors present evidence that DNA polymerase β activity alone is quantitatively correlated with repair replication in HeLa cells. We report here *in vivo* studies which demonstrate a differential sensitivity to aphidicolin between DNA replication and DNA repair (as measured by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) induced unscheduled DNA synthesis (UDS) in 3T3 cells). The concentrations of aphidicolin required for 50% inhibition of UDS and replication are 7.0 and 0.1 μM, respectively. Our data show that the involvement of DNA polymerase α, if any, in MNNG-induced UDS is 70-fold less sensitive to aphidicolin than in DNA replication. (Supported in part by Grant CA23994 from the National Institutes of Health and by the TWU Faculty Research Fund.)

522 POST REPLICATION REPAIR OF UV-DAMAGE IN HERPES SIMPLEX VIRUS. Uma B. Dasgupta and William C. Summers. Dept. of Therapeutic Radiology, Yale University School of Medicine, New Haven, Conn. 06510

Role of recombination in post replication repair of DNA damage in eucaryotes has not been assessed fully. The high recombination proficiency of herpes simplex virus (HSV) together with the fact that recent evidence indicates UV-induced pyrimidine dimers to be recombinogenic in this virus led us to investigate the role of recombination in post replication repair of HSV. Excision defective cells were infected with UV'd HSV in the presence of ^{3}H-thymidine. Resulting labelled herpes DNA was separated from cellular DNA utilizing its high density and was then assayed for presence of Micrococcus luteus endonuclease sensitive sites, which are known to be specific for thymine dimers. These experiments showed that UV'd HSV DNA which was labelled during genome replication contained pyrimidine dimers. Although these results were obtained in excision-deficient host cells which suggests that the label was in progeny DNA, the possibility has yet to be excluded that the dimers are in parental DNA labeled by some unknown virus-induced repair process.

523 THE AFFECTS OF ULTRAVIOLET IRRADIATION ON SIMIAN VIRUS 40 DNA SYNTHESIS. Pamela C. Stacks and Kathleen Hercules. University of California, Los Angeles, Ca. 90024

The response of eukaryotic repair pathways to ultraviolet irradiation, a physical mutagen, has been examined utilizing a viral probe, simian virus 40 (SV40). The small closed, circular genome is contained in a chromatin-like structure and is replicated by host cell enzymes.

Virus-infected cells (CV1 cells of African Green Monkey Kidney) were irradiated (UV doses of 0-3 dimers/genome) during maximal viral DNA synthesis. The infected cells were pulsed with 3H thymidine, viral DNA extracted, and electrophoresed on agarose gels.

A 15 minute pulse of 3H thymidine administered immediately after irradiation indicates that the total incorporation of label into viral DNA as well as the amount of labelled form I DNA (completely replicated, superhelical DNA) present decreases as a function of UV dose.

15 minute pulses of 3H thymidine administered at least 15 minutes post irradiation resulted in fewer labelled form I DNA molecules and these molecules appeared to not contain pyrimidine dimers, a major UV photoproduct (as assayed by T4 UV endonuclease which catalyzes the hydrolysis of the phosphodiester bond 5' to pyrimidine dimers).

524 A SIMPLE AND RAPID SCREENING PROCEDURE FOR TESTING THE SENSITIVITY OF ATTACHED CELLS TO RADIATION AND TO DRUGS, Thomas E. Evans and Helen H. Evans, Case Western Reserve University, Cleveland, Ohio 44106.

We have recently developed a rapid, simple and inexpensive procedure for characterizing newly isolated cell strains as to their growth response to radiation and to drugs. Following treatment of the cells with various doses of radiation, samples are pipetted into column 1 of a 96-well microtiter dish. Medium is added to the remaining wells, and the cell samples are serially diluted through the remaining 11 columns using a multi-tip adjustable volume pipette. After incubation for 4-7 days, the medium is removed, and the cells are fixed and stained. The absorbance of each well is then determined with a microspectrophotometer. Under appropriate conditions, the absorbance is proportional to cell number. For measuring the response of cells to drugs, equal numbers of cells are pipetted into each well, while the concentration of the drug is varied by serial dilution. Each microtiter dish thus can accommodate cells treated with eight doses of radiation or eight different strains treated with 12 drug concentrations. Each dish requires only 20 ml of medium, and its processing takes only a short time. A further advantage is that the large number of dilutions allows a measurement of survival even when an unexpected response is encountered.

(Supported by NIH grant CA 23427 and DOE Contract DE-AC02-7704472)

525 METABOLIC REQUIREMENTS FOR THE RECOVERY OF DNA SYNTHETIC RATES FOLLOWING MUTAGEN INSULT. T.D. Griffiths, P.J. Meechan and D.B. Dahle, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642

Chinese hamster V-79 cells were treated with metabolic inhibitors of DNA or protein synthesis for various intervals of time after exposures of 3.0 to 10.0 $J.m^{-2}$ or treatment with 3 mM methylmethane sulfonate (MMS). After removal of the metabolic block(s) the rate of DNA synthesis was followed by measuring the incorporation of radioactively labeled thymidine into acid insoluble material or by measuring the rate of DNA chain growth as visualized in DNA fiber autoradiographs. For exposures of 3.0 to 5.0 $J.m^{-2}$ a 2.5 or 5.0 hr post-UV exposure with cycloheximide (CHI) or hydroxyurea (HU) was effective in delaying the onset of the recovery in the rate of DNA synthesis. By using concentrations of CHI or HU that inhibit DNA synthesis by a similar amount (70%), but protein synthesis by vastly different amounts (95% for CHI; ~ 0% for HU), it was apparent that the delay in recovery caused by the treatment of the cells with CHI could be accounted for entirely by its inhibitory effect on DNA synthesis. Fiber autoradiographic analysis of cells exposed to 10.0 $J.m^{-2}$ or treated for 1/2 hour with 3 mm MMS indicated that inhibition of protein synthesis had little if any effect on the ability of cells to recover the ability to synthesize DNA segments of normal size. Thus the recovery in DNA synthetic rates following exposure of V-79 cells to UV or MMS does not appear to require de novo protein synthesis and, therefore, does not appear to require the involvement of an inducible DNA repair process. Work was supported by the Department of Energy (Contract No. DE-AC02-76EV03490) and by NIH Grant AG01412.

526 POSTREPLICATION REPAIR IN Escherichia coli K-12, Tzu-chien V. Wang and Kendric C. Smith, Dept. of Radiology, Stanford Univ. School of Medicine, Stanford, CA 94305

If noncoding lesions produced in DNA by chemical carcinogens and radiation are not removed by excision repair, they interfere with normal DNA replication and lead to the production of DNA daughter strand gaps, which are the substrates for postreplication repair. We have studied the genetic control of postreplication repair in uvrB derivatives of E. coli K-12, using far ultraviolet (UV) light as a model agent for producing noncoding DNA lesions. The degree of UV radiation sensitivity of the multiply mutant strains in the uvrB background were: recF recB uvrD lexA, recF recB lexA, recF recB uvrD > recA > recF uvrD lexA > recF recB, recF uvrD, recF lexA > recB uvrD lexA > recB uvrD > recB lexA, lexA uvrD > recB > lexA, uvrD, recF. Analysis of the survival curves of these multiply mutant strains revealed that the recF gene interacts synergistically with the recB, uvrD, and lexA genes in UV radiation sensitization. On the other hand, the recB, uvrD, and lexA genes appear to interact additively with each other. At present, the most plausible interpretation of these results is that there are at least *two independent pathways for postreplication repair, one of them dependent on the recF* gene and the other dependent on the recB, uvrD and lexA genes. (Research supported by Public Health Service research grant CA-02896 and research program grant CA-10372 from the National Cancer Institute.)

527 PERSISTENCE OF METHYLATED PURINES IN CULTURED HUMAN FIBROBLAST DNA, Andrew S. C. Medcalf and P. D. Lawley, Pollards Wood Research Station, Buckinghamshire, England. Pollards Wood Research Station, Inc. of Cancer Research, Buckinghamshire, England.
The persistence in DNA of the three major methylated purines produced by the potent methylating carcinogen N-methyl-N nitrosourea (MNUA), 7-methylguanine, O^6-methylguanine and 3-methyladenine, was studied in several cultured human cell strains. These strains included cells derived from normal donors and those from patients with ataxia telangiectasia and xeroderma pigmentosum (both cancer prone syndromes and x-ray and UV-sensitive, respectively). The results indicate that the individual half-lives of the three bases in DNA are similar in all cell lines investigated, at a given dose. It was observed, however, that the efficiency of removal of O^6-methylguanine from DNA was much higher after treatment of cells with a low dose of MNUA than after treatment with a high dose. This may be illustrated by the fact that the half-life of O^6-methylguanine produced in DNA by a low dose of MNUA (about 0.1 mM) is about 1 hr which compares with a corresponding estimate of about 30 hr after treatment of cells with a high dose of MNUA (about 0.5 mM). A dose dependent efficiency of removal from DNA was not observed for the other two methylated purines studied. Since alkylation of the O-6 atom of guanine is thought to be important for the induction of mutations and cancer by alkylating carcinogens, these findings suggest that humans may be very resistant to the carcinogenic effects of the low levels of methylating carcinogens to which they are exposed in the environment.

528 BIOLOGICAL EFFECTS OF ENU-INDUCED PERSISTENT DNA-DAMAGE IN DENSITY-INHIBITED SYRIAN HAMSTER EMBRYO CELLS, Ad J.de Kok, Antonius A.van Zeeland, Ad D.Tates, Jo W.I.M. Simons and Leo den Engelse, State University of Leiden, Leiden, The Netherlands.
Many carcinogens induce persistent lesions in the DNA. This indicates that these lesions are chemically stable and not sensitive to attack by DNA repair-enzymes. Little is known about the biological effects of such lesions, although they might be important with respect to(human) carcinogenesis. Therefore we decided to analyse the effects of persistent lesions on cytotoxicity, chromosome damage, mutation and transformation in an in vitro cell system. Here we report experiments in which density-inhibited SHE cells are treated with ENU and subsequently released from density-inhibition at 0, 3, or 6 days thereafter to determine the biological effects of residual DNA damage. First we present data about the inhibition of semi-conservative DNA synthesis in the density-inhibited state as determined by autoradiography or BrdUrd-incorporation and subsequent analysis of the DNA on CsCl-gradients.With the last method the cellturnover over stationary-phase periods of up to 6 days was determined. Secondly, with regard to the biological parameters, the data obtained sofar indicate that a 6-day period of stationary-phase recovery after ENU treatment led to an enhanced cell-survival, whereas frequencies of SCEs and micronuclei gradually decreased from high values at day 0 to lower values at day 6.
Since the number of phosphtriesters, as determined by alkaline sucrosegradient sedimentation, remains constant over this period, it is indicated that these lesions are not the major cause of celldeath, SCEs and micronuclei in this system.

529 CORRELATION OF REACTION KINETICS OF MONOFUNCTIONAL ALKYLATING AGENTS WITH INDUCTION OF MUTATIONS,SCEs AND CHROMOSOME ABERRATIONS. J.W.I.M. Simons and A.T. Natarajan. Department of Radiation Genetics and Chemical Mutagenesis, University of Leiden, Holland.

Monofunctional agents (MMS, DMS, EMS, MNU and ENU) were compared in a) cell killing, b) induction of mutations, c) induction of SCEs and d) induction of chromosome aberrations.
All these biological endpoints were determined simultaneously in V-79 Chinese hamster cells, which had been treated for one hour at 37 degree C. Subsequently the results were compared with the chemical reaction pattern of the compounds. As a parameter for this reaction pattern the Swain-Scott s factor was used, which expresses the dependence of the reaction rate on the nucleophilicity of the receptor. Alkylating agents with a low s value will produce a relatively high ratio of O_6 : N_7 alkylation of guanine and are supposed to be very effective in the induction of gene mutations.
The concentrations needed to obtain 90% killing differ widely for these compounds and are not correlated with the s factor.
The ratios of the other parameters (as calculated for the linear parts of the dose-response relationships) show that with decreasing s factor relatively more mutations are induced than chromosome breaks or SCE. This indicates that the alkylations which lead to SCE differ from those, which lead to point mutations and is in agreement with the notion that O_6- alkylation of guanine is the adduct which is primarily responsible for point mutations.

530 HERPES SIMPLEX VIRUS AS A PROBE FOR REPAIR OF DNA DAMAGED BY 4,5',8-TRIMETHYLPSORALEN PLUS LIGHT, Jennifer D. Hall and Karen Scherer, University of Arizona, Department of Cellular and Developmental Biology, Tucson, AZ 85721

4,5'8-trimethylpsoralen (psoralen) reacts with DNA in the presence of 360 nm light to produce both monoadducts and diadducts (crosslinks). We have investigated the repair of psoralen-damaged herpes simplex virus (HSV-1) during infection of human fibroblasts. Cells infected with two or more damaged viral genomes repaired virus more efficiently than singly infected cells (multiplicity reactivation). These results suggest that recombination between damaged viral genomes in multiply infected cells may be an important mechanism for repair of psoralen damage. The effect of psoralen damage on the yield of temperature resistant viral recombinants in cells infected with pairs of temperature sensitive mutants was investigated. Increased recombination frequencies were observed following psoralen treatment of the mutant virus providing further evidence that genetic recombination is involved in repair of psoralen-damaged herpes DNA. Finally, measurement of the frequency of crosslink formation suggests that DNA containing crosslinks can be repaired in multiply-infected cells but not in singly infected cells, whereas DNA containing monoadducts is repaired in both cell types. This observation is consistent with the idea that crosslinked DNA requires genetic recombination for repair, but monadducts may be repaired by alternative mechanisms.

531 THE EFFECTS OF BENZPYRENE DERIVATIVES ON DNA REPLICATION, Liebe F. Cavalieri, Izumi Yamaura, Michael Lockhart and B. Rosenberg, Sloan-Kettering Institute, Rye, NY 10580

DNA's treated with benz(a)pyrene diol epoxides (BP) to produce 1-25 adducts per molecule have been used as templates to study the effects of adducts on misincorporation and chain termination during DNA replication. We have shown that syn and anti adducts formed in polydeoxynucleotides do not result in misincorporation of erroneous nucleotides in an in vitro system using AMV DNA polymerase. Termination of synthesis was studied using φX174 DNA containing similar adducts. The modified template was annealed to a 392-base primer obtained by treating φX174 rf DNA with Hpa I. Synthesis was markedly decreased and termination occurred at each adduct site when using either E coli Pol I or its large proteolytic fragment. Both the syn and anti adducts decrease the amount of DNA synthesis to the same extent. It is likely that the BP adducts directly block DNA replication, since no mispairing was observed in the experiments with AMV polymerase; however, since the polymerase used in the termination studies contains an editing function, unlike AMV polymerase, we are currently investigating deoxynucleotide triphosphate turnover in the system to test the possibility that "idling" at adducted sites may account for termination. Since anti BP is much more carcinogenic than the syn compound we infer that the two may act differently in vivo. To study this question we have developed an in vivo system in which SV40 virus is modified with the syn or anti BP derivatives. Cells are co-infected with the treated virus and a helper virus containing a viable deletion. Early progeny DNA is isolated and quantified by gel electrophoresis, using the deletion to distinguish helper progeny from the progeny of interest. We are now investigating chain termination in this system.

532 CHARACTERIZATION OF A REPLICON-CONTROLLED FUNCTION REQUIRED IN THE INITIATION OF THE SOS PATHWAY IN *E. coli*. R. Devoret, Enzymologie, CNRS, 91190 Gif-sur-Yvette, France.

Inducible error-prone repair of bacteriophage λ as well as prophage λ induction can be promoted in the absence of direct exposure of the host-cell to a detrimental agent. It is sufficient that UV-damaged DNA be introduced into the host-cell to cause indirect induction of the two afore-mentioned SOS functions. For instance, a UV-damaged replicon such as the F sex factor or phage P1 causes indirect induction of prophage λ.

We have recently characterized (Devoret et al, 1981) the portion of the F replicon responsible for indirect prophage induction. The portion encompasses a few genes around the origins of replication. The exact location of the site governing indirect prophage induction has been determined by deletion mapping. Furthermore, mutations have been isolated that make a transferred F replicon cause indirect prophage induction in the absence of UV-damage (Bailone et al., 1981). Such mutations are "constitutive" for indirect prophage induction.

Prophage induction results from the cleavage of the prophage repressor by RecA protein (Roberts et al., 1978). We have provided indirect evidence that RecA protease (RecAp) is but a fraction of the population of RecA molecules (Moreau et al., 1980). In vivo, conversion of RecA molecules into RecAp requires not only the factors identified by Craig and Roberts (1980) in vitro but also the replicon-controlled function we have characterized.

533 SPECIES VARIATION IN DNA DAMAGE CAUSED BY THE PANCREATIC CARCINOGEN, N-NITROSOBIS-(2-OXOPROPYL) AMINE. Joanne Zurlo, Daniel S. Longnecker and Thomas J. Curphey, Dartmouth Medical School, Hanover, NH 03755. N-Nitrosobis(2-oxopropyl)amine (BOP) has been shown to induce pancreatic ductal cell tumors, as well as liver tumors, in the Syrian golden hamster. However, in the rat, while BOP does induce liver tumors, it is not a carcinogen for pancreas. The object of the present study was to assess DNA damage by BOP in the pancreas and liver of these two species in an attempt to relate the extent and persistence of DNA damage to carcinogenesis. Male Syrian golden hamsters or Lewis rats were injected ip with BOP. At the time of sacrifice, the livers and pancreases were removed, nuclei were prepared, and the DNA damage was measured by the alkaline elution technique. Damage in both rat and hamster liver and pancreas was studied as a function of dose and time. Hamster pancreatic DNA, examined one hour after injection, is moderately damaged at doses of 3-20 mg/kg body weight and more severely damaged at a dose of 40 mg/kg. DNA damage in hamster pancreas produced by 20 mg/kg of BOP is evident 4 hours after injection, but not at 24 hours. At the same BOP dose levels, pancreatic DNA from rats exhibits only slight damage which is not dose dependent. The extent of pancreatic DNA damage produced in one hour by 100 and 200 mg/kg of BOP in the rat was roughly equivalent to that produced by one-tenth those doses in the hamster. However, DNA damage in rat pancreas persisted for 24 hours. Liver DNA from rat was shown to exhibit significantly more damage than that from hamster at lower (10-40 mg/kg) doses of BOP. It does appear, however, that liver DNA damage is more persistent in hamster than in rat. These results suggest that the carcinogenic activity of BOP correlates with organ and species variation in metabolism and/or DNA repair. Supported by NIH grants-CA 17843 and F32 CA 06855

534 FORMATION AND REMOVAL OF ETHYL DERIVATIVES IN DNA FROM SEVERAL TISSUES FROM ETHYLNITROSOUREA-TREATED BD IX RATS, *B. Singer*, W.J. Bodell†, S. Spengler**
Univ. of Cal., Berkeley 94708, San Francisco 94143†*

10 day old BD IX rats, known to selectively develop brain tumors after a single administration of ethylnitrosourea, were studied. Seven derivatives in three DNAs were quantitated 1,25,50 and 75 h after intraperitoneal injection of [^{14}C] EtNU. Data are shown in the Table below:

Position of Ethylation	Liver DNA 1 h after EtNU % of Total Ethylation	Repair Capacity[a] Overall 1 - 75 h Liver	Brain	Other Tissues
e^7G	14.2	+	+	-
e^6G[b]	5.7	+++	+	+
e^3A[b]	2.7	+	+	+
e^2T	7.2	++	+	+
e^4T	2.4	++	-	+
e^2C	1.4	+	+	±
Phosphate	∿63.	±	±	±

[a] The number of pluses is subjective but based on quantitative data. +++ denotes very fast or active removal. Fewer pluses are for less efficient removal. ± and - are for poor or undetectable removal. In general, the rates of removal of most derivatives are bi- or multiphasic with the greatest removal at short times after treatment.

[b] These values reflect the rapid initial loss of e^6G and e^3A from the liver.

We have demonstrated that not only cultured human cells [Nucleic Acids Res 6, 2819 (1979)] but whole mammals have multiple repair enzymes which can remove O-ethyl pyrimidines and ethyl purines from DNA. The efficiency of removal varies with time and tissue. Liver is ethylated more than other tissues but also is generally more efficient in repair. In the brain the relative persistance of the promutagens e^6G, e^2T, e^4T and e^2C may contribute to oncogenesis.

535 QUANTITATION OF N-HYDROXY-2-ACETYLAMINOFLUORENE MEDIATED DNA ADDUCT FORMATION AND THE SUBSEQUENT REPAIR IN PRIMARY RAT HEPATOCYTE CULTURES, Paul C. Howard, Frederick A. Beland, and Daniel A. Casciano, National Center for Toxicological Research Jefferson, AR 72079, and University of Arkansas for Medical Sciences, Little Rock, AR 72205

Primary cultures of rat hepatocytes were exposed to [ring-^{3}H]-N-hydroxy-2-acetylaminofluorene (N-OH-AAF) for 4 hours, the DNA and RNA nucleoside adducts were isolated and then identified by HPLC. The DNA adducts were shown to be N-(deoxyguanosin-8-yl)-2-acetylaminofluorene (dG-C8-AAF), N-(deoxyguanosin-8-yl)-2-aminofluorene (dG-C8-AF), and 3-(deoxyguanosin-N^2-yl)-2-acetylaminofluorene (dG-N^2-AAF), while the RNA adducts were N-(guanosin-8-yl)-2-acetylaminofluorene, and N-(guanosin-8-yl)-2-aminofluorene. The removal of the RNA adducts was measured for 38 hours following the cessation of exposure of the hepatocytes to N-OH-AAF. dG-C8-AAF was rapidly removed from the DNA and had a half-life of approximately 10 hours; dG-N^2-AAF and dG-C8-AF remained constant for 14 hours, and then were removed at a slow rate. The dG-C8-AAF adduct initially composed about 60% of the total DNA adducts of primary hepatocytes in contrast to the 20% found in liver *in vivo*. When suspensions of hepatocytes were simultaneously exposed to N-OH-AAF and the deacylase inhibitor, paraoxon (10^{-5}M), for 2 hours, the relative yield of the adducts did not change. This suggests that the low yield of dG-C8-AF may be due to partial loss of *N,O*-acyltransferase during hepatocyte isolation and *in vitro* cultivation, rather than alteration of the deacylase activity. The formation of the three DNA adducts and the different rates of repair indicate that primary cultured rat hepatocytes may be a valuable system to study initiation of liver carcinogenesis by N-OH-AAF.

536 DIFFERENTIAL REACTIVATION OF UV DAMAGE TO INDUCIBLE THIONEIN GENES IN NORMAL AND REPAIR DEFICIENT HUMAN CELLS, C. E. Hildebrand and G. F. Strniste, Genetics Group, Los Alamos Scientific Laboratory, Los Alamos, NM 87545

The ubiquitous, low molecular weight, thiol-rich, metal-binding protein, metallothionein (MT) can be induced in normal human skin fibroblasts (NF) and xeroderma pigmentosum (XP) cells in culture during exposure to $ZnCl_2$ at a maximal subtoxic level of 200 μM. Both cell types show similar induction kinetics: during continuous exposure to 200 μM Zn^{2+} the cellular MT synthesis rate rises within 7 h from a low, basal rate to a maximal rate at least 50-fold > basal rate. Induction of MT synthesis is inhibited by actinomycin D (5 μg/ml). Irradiation of these cells with FUV caused inactivation of induction of MT synthesis: i.e. exposure to FUV followed by induction with Zn^{2+} resulted in a dramatic UV-dose-dependent decrease in the MT synthesis rate measured 8.5 h postinduction. The UV-sensitivity of MT induction appeared to be greater in XP cells than in NF cells. However, this apparent difference in sensitivity of MT induction could be explained (a) by the differential repair capacities of normal and XP cells and (b) by considerations of the kinetics repair of FUV-induced lesions superimposed on kinetics of MT induction. Liquid holding recovery experiments demonstrated that NF cells possess the capacity to reactivate MT gene expression completely while no reactivation is observed in XP cells. The implications of these findings are discussed with regard to (a) UV transcriptional mapping of inducible gene functions and (b) application of this inducible gene system as a new method for assessing repair capacities for various types of DNA-lesions produced by chemical and physical agents. (This work was supported by the US Department of Energy.)

537 THE DISTRIBUTION OF DNA DAMAGE IN 10T1/2 CELLS TREATED WITH N-METHYL-N-NITROSOUREA (MNU) DURING S PHASE, M. Cordeiro-Stone, M.D. Topal, and D.G. Kaufman, University of North Carolina Medical School, Chapel Hill, N.C. 27514.

Knowledge of the target specificity involved in covalent binding of chemical carcinogens to DNA is crucial to understanding the processes of mutagenesis and carcinogenesis. Thus, using MNU, we methylated C3H/10T1/2 clone 8 cells synchronized in S phase to study the suggested specificity of alkylation of DNA at sites of replication (W.K. Kaufmann and D.G. Kaufman (1976) Proc. Am. Ass. Can. Res. (Abstract) 17, 133). Replication fork DNA was separated from bulk cell DNA by restriction of the genome followed by isolation on nitrocellulose (NC) filters. Thymidine incorporated into DNA for 30 minutes was found to be enriched 3-6 fold (over the range of restriction digests studied) in this NC bound fraction compared to that observed with uniformly labelled DNA. Similarly, when DNA treated with 0.018-0.040 mM [^{3}H] MNU for 30 minutes was analyzed by this method, the distribution of methyl groups was also 3-5 fold enriched in the NC fraction over that expected from random methylation. No inhibition of DNA synthesis was detected in this range of MNU concentrations and toxicity was less than 30%. Contributing factors to this greater susceptibility of the fork region to methylation may include alkylation of the single-strand region of the lagging side of the replication fork, modification of DNA precursor molecules which are then incorporated (M.D. Topal et al. (1980) ICN-UCLA Symp., 19, 728-733; and see M.S. Baker and M.D. Topal, these abstracts) or possibly both pathways. Experiments to determine the strand specificity, if any, of replication fork alkylation are in progress. Supported in part by grants from the NIH (CA 20658 and RCDA CA 00431) to D.G.K., (GM 24798 and CA 28632) to M.D.T., and training grant ES 07017 (M.C.S.).

538 CELL SPECIFIC DIFFERENCES IN ALKYLATION, REPAIR AND REPLICATION IN HEPATOCARCINOGENESIS J.A. Swenberg, M.A. Bedell, K.C. Billings and J.G. Lewis, Chemical Industry Institute of Toxicology and Duke University, Research Triangle Park and Durham, NC 27709.

A variety of liver tumors which arise from specific cell types can be induced by chronically administering chemical carcinogens. Previous studies on the importance of DNA alkylation in hepatocarcinogenesis have utilized whole liver. We have now evaluated the extent and persistence of DNA alkylation in hepatocytes and non-parenchymal cells (NPC) isolated by centrifugal elutriation following repeated oral administration of 1,2-dimethylhydrazine (DMH), a regimen that only induces tumors of the non-parenchymal cells. While initial levels of O^6-methylguanine (O^6-MG) in NPC were 73% those of hepatocyte DNA, significant differences in DNA repair resulted in a 28-fold greater O^6/N-7 MG ratio in NPC 24 hours after a second daily dose of DMH. Metabolic incorporation of ^{14}C into normal purine bases suggested an increased rate of cell turnover in the NPC. To better evaluate this, 3 rats were exposed to 3 mg DMH/kg/day in their drinking water for 8 days. Following this a 300 mg pellet of BrdU was implanted subcutaneously and ^{3}H-thymidine was administered IP hourly. The animals were killed 7 hours after pellet implantation. DNA of hepatocytes and NPC were banded using CsCl gradients. Cell replication and repair were evaluated by comparing radioactivity in heavy and light DNA, respectively. Cell replication was 11.8 ± 3.0 times greater in the NPC. Conversely, repair was primarily restricted to hepatocytes. Thus, the persistence of O^6-MG and the level of cell replication were much greater in the target cell than in the non-target cell population. These data also suggest that the loss of O^6-MG from NPC may reflect cell death rather than repair.

539 THE EFFECTS OF ETHYLATING AGENTS ON DNA SYNTHESIS IN VITRO: IMPLICATIONS FOR THE MECHANISM OF CARCINOGENESIS, Barbara H. Rosenberg and Joan Deutsch, Sloan-Kettering Institute, Rye, NY 10580

A highly carcinogenic ethylating agent, ethylnitrosourea (ENU), and a weakly carcinogenic one, diethylsulfate (DES), have been compared with respect to their effects on DNA. Their total reactivity with DNA is roughly the same but DES produces about six times more unstable ethylated bases, which are gradually lost spontaneously under physiological conditions. The different rates of loss of the four bases have been studied using polydeoxynucleotides. Spontaneous strand breakage following base loss is slow, lagging more than a week behind base loss at 37°C; ultimately, DES results in much more spontaneous strand breakage than ENU. In DNA synthesis in vitro, using AMV polymerase, no nucleotides are incorporated opposite missing bases in the template; when the template contains ethylated bases that are impaired in their ability to form specific hydrogen bonds, purine-pyrimidine (but not pu-pu or py-py) mispairing can occur and mismatched nucleotides are incorporated into the daughter strand. ENU ethylates only about 3.5 times more sites with mispairing potential than DES, but it produces many more ethyl phosphotriesters. We deduce that spontaneous base loss and strand breakage are not important in carcinogenesis by ENU, and that mispairing by ethylated bases is probably also unimportant; phosphotriester formation or some specific type of damage incurred by attempted repair of one of the oxygen sites preferentially ethylated by ENU appear most likely to be critical events in carcinogenesis by ENU.

540 UNSCHEDULED DNA SYNTHESIS (UDS) IN HUMAN SKIN IN RESPONSE TO DNA-DAMAGING THERAPEUTIC AGENTS: UVB AND PSORALEN PLUS UVA, Simone C. Bishop, Stanford University, Stanford, CA

DNA-damaging agents: UVB and systemic psoralen plus UVA (PUVA) are used in therapy of hyperproliferative skin diseases such as psoriasis. The ultraviolet light used in PUVA emits DNA-damaging UVB (Brit. J. Derm. 1979 -101,399) as well as UVA which photoactivates the covalent binding of psoralen to DNA. These DNA-damages presumably delay DNA semi-conservative replication and reduce proliferation of the involved psoriatic skin. We have been concerned with establishing the nature of DNA damage and the adequacy of repair processes in the uninvolved (normal) skin of psoriatic patients which is also exposed to the DNA-damaging agents during treatment and in which an increased incidence of skin cancers has been reported - (New England J. Med. 300,809,1979). We detect UDS indicative of DNA damage in the uninvolved skin of psoriatic patients receiving PUVA and show that this activity is elicited by UVB. No activity is detected when UVB is eliminated from the light by filtration during treatment and the skin exposed to pure UVA and psoralen. Psoralen plus UVA treatment does not interfere with the UVB-stimulation of UDS. In human skin in vitro, UVB stimulates UDS efficiently indicating adequate repair of DNA damage and no inhibition of semi-conservative replication is observed. Psoralen plus UVA elicits low levels of UDS and semi-conservative synthesis is inhibited. This inhibition may reflect accumulation of unrepaired DNA damage.

541 HUMAN CELL-MEDIATED POLYCYCLIC AROMATIC HYDROCARBON CYTOTOXICITY, MUTAGENICITY, AND DNA BINDING. A. E. Aust, V. M. Maher, and J. J. McCormick, Carcinogenesis Laboratory, Michigan State University, East Lansing, MI 48824.

We have developed a human cell-mediated metabolic activation system for quantitating the biological effects of carcinogenic polycyclic aromatic hydrocarbons (PAH). In the absence of an exogenous source of metabolic activation, human skin fibroblasts show no cytotoxic response to benzo(a)pyrene (B(a)P) or 7,12-dimethylbenzanthracene (DMBA). However, when we used human fibroblasts from normal individuals or xeroderma pigmentosum (XP) patients as target cells in co-cultivation with epithelial cell lines derived from human carcinomas, we observed a concentration-dependent loss of colony-forming ability by the target fibroblasts with B(a)P or DMBA. Since XP cells are deficient in excision repair of DNA adducts formed by reactive metabolites of PAH, they are very sensitive for detecting the biological effects of such agents and were used to determine optimal conditions for co-cultivation of metabolizing cells and target cells. Using this system we observed a concentration-dependent decrease in colony-forming ability and corresponding increase in frequency of 6-thioguanine resistant mutant colonies in XP target cells co-cultivated with metabolizing cells and either B(a)P or DMBA. There is a dose-dependent increase in the number of DNA adducts formed after B(a)P treatment. HPLC analysis of DNA adducts formed by exposure to tritiated B(a)P indicates that the predominant adduct is formed from deoxyguanosine reacting with the anti isomer 7,8-dihydrodiol-9,10-epoxide of B(a)P. (Supported by D.H.E.W. grant ES07076 from the N.I.E.H.S. and grant R805563010 from the Environmental Protection Agency.)

542 REPAIR OF HYDROCARBON ADDUCTS AND TUMOUR INDUCTION IN MOUSE TISSUES, Peter J. Abbott and Maurice M. Coombs, Imperial Cancer Research Fund, London WC2A 3PX, England

The formation and repair of DNA adducts of the carcinogen, 15,16-dihydro-11-methylcyclopenta-[a]-phenanthrene-17-one, have been studied in three mouse tissues. After a single intramuscular dose of the carcinogen (120 mg/kg), binding to DNA was twice as high in the liver, the tissue resistant to tumour formation, than in skin or lung, the tissues susceptible to tumour formation by this carcinogen. Detailed analysis by HPLC of the adducts formed in each of the three tissues revealed no major qualitative differences in the eight adduct peaks found. In vivo removal of the labelled adducts was studied over a 14 day period following initial treatment. In skin and lung, active removal of the major adducts could not be measured above the normal rate of tissue turnover. By contrast, liver adducts were removed rapidly with a half-life of approximately three days. One minor adduct present in both skin and liver was removed more rapidly than the major adducts. The results suggest that the persistence of carcinogen-DNA adducts may be related to tissue specific carcinogenesis by this carcinogenic hydrocarbon and that DNA repair have a role in modifying this effect.

Chromosomal Instability

543 DNA DOUBLE STRAND BREAKS INDUCED IN HUMAN CELLS DURING UVL REPAIR. Matthews O. Bradley. Merck Institute for Therapeutic Research, West Point, PA 19486

Double strand breaks (DSBs) are introduced into E. coli DNA during the repair of ultraviolet light (UVL) damage in wild type but not in UVR^- strains (Bonura, T. and Smith, K. 1975. Photochem. Photobiol. 22: 243-248). To determine whether repair induced DSBs also appear in human cells, $[^{14}C]\cdot TdR$ labeled IMR-90 normal human embryonic fibroblasts were irradiated with 0, 100, and 300 $J\cdot m^{-2}$ of UVL, and then either chilled and harvested immediately or incubated at 37°C for various times up to 51 hours before assay. DSBs were detected by neutral filter elution (Bradley, M.O. and Kohn, K.W. 1979. Nucleic Acids Res. 7: 793-804) which measures the rate at which DNA double strands elute through a 2 μ polycarbonate filter at pH 9.6; this rate is proportional to X-ray dose and therefore the number of DSBs. The results show that repair incubation causes a time and dose-related increase in the rate of pH 9.6 elution and therefore in the number of DNA DSBs. DSBs begin to appear by 3 hours after irradiation and continue to increase up to 51 hours. Since most of the cells remain attached to the plates and exclude trypan blue the DSBs are not likely to be a secondary result of cell death. By contrast, Xeroderma pigmentosum group A cells (XPA) did not induce DSBs after UVL even though they are more sensitive to its cytotoxicity. Since normal human cells are excision competent and form DNA DSBs after UVL, whereas XPA cells do neither, excision repair is implicated as the mechanism for DNA DSB formation after UVL. The cellular effects of even one excision gap overlap that results in an unrepaired DNA DSB might include cytotoxicity, cytogenetic changes, and deletion mutations.

544 REPAIR OF GAMMA-AND BLEOMYCIN-INDUCED DNA DAMAGE IN ATAXIA TELANGIECTASIA PATIENTS AND FAMILIES. K.Y. Hall, R.A. Gatti and R.L. Walford. Dept. of Pathology. UCLA School of Medicine. Los Angeles 90024.

Ataxia telangiectasia (AT) is an autosomal recessive human disease, characterized by progressive neurological deterioration, immunological deficiencies, premature aging and an increased frequency to cancer (primarily lymphoidal). While heterozygotes lack clinical features of the disease, they share a susceptibility to cancer as well as an increased level of chromosomal abnormalities. Cells from AT patients are known to have an increased sensitivity to the effects of ionizing radiation and radiometric drugs such as bleomycin. In the present study, freshly isolated lymphocytes from six families with affected children were analyzed for excision repair capacity of gamma-and bleomycin-induced DNA damage. These results were compared to PHA responses, T/B cell levels and other immunological parameters. A decrease of excision repair, as measured by ^3H thymidine uptake of gamma-induced DNA damage, was found in affected children (homozygotes), as well as parents (heterozygotes) and a proportion of the siblings. Excision repair of damage induced by bleomycin, while decreased, was not statistically significant in either homozygotes or heterozygotes. PHA responses averaged 60% of controls in homozygotes and were normal in both obligate heterozygotes and siblings. This evidence suggests that the observed decrease in excision repair capacity in both homozygotes and heterozygotes reflects a distinct and separate mechanism from control of mitogenic stimulation and T cell levels. Supported in part by USPHS research grant AG-00790.

545 REPAIR OF BLEOMYCIN-DAMAGED DNA BY HUMAN FIBROBLASTS, Myra M. Hurt, Arthur L. Beaudet and Robb E. Moses, Baylor College of Medicine, Houston, Texas 77030. Bleomycin (BLM) is a glycopeptide antibiotic currently used in cancer chemotherapy. The main target of the drug is DNA resulting in base loss (apurinic/apyrimidinic sites) and strand scission. The DNA-damaging activities lead to activation of a specific repair pathway. The ability of cells to repair the specific lesions caused by BLM was investigated in cell lines isolated from patients with diseases caused by apparent DNA repair defects. The diseases ataxia telangiectasia (AT), Bloom Syndrome (BS), Cockayne Syndrome (CS), Fanconi's Anemia (FA) and xeroderma pigmentosum (XP) were those selected for study. We examined in vivo the repair of prelabeled DNA after BLM exposure by alkaline isokinetic gradient centrifugation. A fall in molecular weight of DNA after exposure to BLM is observed by this technique. When the drug is removed, the DNA reforms rapidly to high molecular weight. This fall in molecular weight upon exposure to BLM is observed in all lines except some XP lines. Cell lines from some of the XP complementation groups were found to have persistent low molecular weight DNA fragments which disappeared upon exposure to BLM.

546 SURVIVAL OF ^{60}Co-IRRADIATED HERPES SIMPLEX VIRUS IN HUMAN DIPLOID FIBROBLASTS, Glen B. Zamansky and John B. Little, Harvard School of Public Health, Boston, MA. Viruses have recently become an important tool for studying DNA repair, mutagenesis and recombination in mammalian cells. Most investigations have emphasized the effects of ultraviolet light on the recovery of infectious virus. This study was undertaken in order to determine the extent to which herpes simplex virus (HSV) may be utilized to investigate the repair of DNA damaged by ionizing radiation.

Cell strains derived from normal controls and patients with a broad range of diseases associated with DNA repair deficiencies were utilized. The survival curves of HSV treated under conditions which minimized the indirect effects of irradiation could generally be resolved into two components. Thus far, infection of cells derived from individuals with ataxia telangiectasia, hereditary retinoblastoma, Gardner's syndrome, progeria and Fanconi's anemia resulted in D_o's which were not significantly lower than those of controls. Virus treatment under conditions which permitted damage from direct and indirect effects of irradiation resulted in greatly decreased virus recovery and single component survival curves. No significant differences in D_o's have been observed under these conditions. Experiments examining the reactivation of HSV in cells pretreated with ultraviolet light or ^{60}Co irradiation have also been initiated. The significance of our results with respect to cellular survival studies and investigations utilizing other viruses will be discussed.

547 REPAIR OF DNA DOUBLE STRAND BREAKS IN ATAXIA TELANGIECTASIA AND FANCONI FIBROBLASTS, Thérèse M. Coquerelle and Karl F. Weibezahn, Kernforschungszentrum Karlsruhe, Institut für Genetik und für Toxikologie, Postfach 3640, D-7500 Karlsruhe 1, F.R.G.

Ataxia telangiectasia and Fanconi anemia are autosomal recessive diseases of man with an predisposition to cancer and an elevated sensitivity to various mutagenic agents as measured by decreased cell survival and increased level of both, spontaneous and induced chromosome aberrations. The precise biochemical defect has yet to be identified, but clinical and experimental data support a deficiency of DNA repair in both diseases. We have investigated whether the step which is deficient, is required for the repair of DNA double strand breaks.

Using the neutral filter elution technique the repair of double strand breaks was studied in Ataxia telangiectasia and Fanconi fibroblasts from different patients. Two strains were reduced in the rate of break rejoining: AT2BE (CRL 1343) which has earlier been reported as excission deficient (1) and cells from a Fanconi patient (1424 Berlin collection) which has been shown earlier to have reduced DNA ligase activity (2). Furthermore, the rate of repaired double strand breaks in these two strains never reached the same plateau level as in normal fibroblasts. Thus, the enzymes deficient in these two patients participate in the repair of DNA double strand breaks.

(1) Paterson, M.C., P.J. Smith, N.T. Beck-Hansen, B.P. Smith and B.M. Sell. Proceedings of the 6th International Congress of Radiation Research, 1979, Tokyo, p. 484

(2) Hirsch-Kauffmann, M., M. Schweiger, E.F. Wagner and K. Sperling, Hum. Genet. 45, 25-32 (1978)

548 SCEs AND CHROMOSOMAL INSTABILITY: DIFFERENTIAL RESPONSE OF HUMAN RETINOBLASTOMA AND NEUROBLASTOMA CELLS TO MITOMYCIN-C, Beverly S.Emanuel, Gloria Balaban and Fred Gilbert, University of Pennsylvania, Philadelphia, PA 19104

We have examined spontaneous and Mitomycin-C (MMC) induced sister chromatid exchange (SCE) frequencies in human retinoblastoma and neuroblastoma cell lines. The spontaneous SCE frequency in cells from both tumors is similar to the frequency in normal human diploid cells. In normal cells a dose of 25 ng/ml MMC causes a 2-3 fold increase in SCE/cell. In neuroblastoma and retinoblastoma cells this dose causes a 3-5 fold increase while in retinoblastoma only,one sees in addition to the elevated SCE frequency marked chromosomal damage and rearrangement in the few surviving cells. The damage is of the type seen in the chromosomal fragility syndromes (quadriradials and breaks). A lower dose of MMC elicits a similar response.

We have examined cells from both tumor lines to determine if chromosomal fragility can be elicited by MMC alone. The results indicate that the retinoblastoma cells exhibit much greater sensitivity to MMC induced chromosomal damage than do the neuroblastoma cells. Thus, while both cell types respond to MMC exposure with an enhanced induction of SCEs, the retinoblastoma cells also exhibit chromosomal fragility. This sensitivity of retinoblastoma cells to MMC supports previous suggestions from this laboratory and others of the presence of a genetically determined susceptibility to chromosomal damage for this disorder, i.e. chromosome 13 breaks in direct tumor preparations and radiosensitivity of skin fibroblasts from retinoblastoma patients.

549 EFFECT OF A CARCINOGEN AND A PROTEASE INHIBITOR ON CHROMOSOME BREAKAGE IN FANCONI ANEMIA: RELEVANCE TO CARCINOGENESIS. Arleen D. Auerbach, Barbara Adler, and R.S.K. Chaganti. Memorial Sloan-Kettering Cancer Center, New York, New York 10021.

Fanconi anemia (FA) patients show increased chromosome breakage in cultured cells, and are at greatly increased risk for development of cancer. Recently, Kinsella and Radman (PNAS 77: 3544, 1980) reported that antipain, a protease inhibitor and anti-carcinogenic agent, inhibited MNNG-induced chromosomal aberrations (which are similar in type to aberrations seen in FA), and speculated that chromosomal rearrangement is a rate-limiting step in carcinogenesis. We have studied the effect in two sisters (FA1 and FA2) of antipain on chromosome breakage induced by diepoxybutane (DEB), another carcinogenic alkylating agent to which FA lymphocytes are especially susceptible (Auerbach et al., Pediatr. 67, 1981). FA2 had a lymphocyte dimorphism, which has persisted in 4 separate studies over two years; only 10% of her cells showed the sensitivity to DEB compared to the almost 100% of cells from other FA patients. Our results (Table) show that antipain did not alter the rate of spontaneous or DEB-induced chromosome breakage. We suggest that carcinogenesis in FA may not always be based on the chromosome instability. For example, myeloid leukemia, the tumor most often found in these patients, may be related to an intrinsic defect in granulopoiesis reported in in vitro studies of myeloid differentiation in some patients.

Table: Breaks/Cell in FA Lymphocytes

	Untreated	Antipain (50µg/ml)	DEB (0.1µg/ml)	DEB and Antipain
FA1	0.33	0.29	13.0	10.56
FA2	0.03	0.02	1.59	1.70

550 CLASTOGENIC ACTIVITY FROM BLOOM's SYNDROME FIBROBLAST CULTURES, Ingrid EMERIT*and Peter A. CERUTTI‡, *Institut Biomédical des Cordeliers, Université Pierre et Marie Curie, Paris, France - ‡ Swiss Institute for Experimental Cancer Research, Epalinges/Lausanne, Switzerland.

Media from cultures of fibroblasts of six patients with the autosomal recessive disease Bloom's Syndrome (BS) and from four normal fibroblast (NF) strains were analyzed for clastogenic activity towards phyto-hemagglutinin stimulated human blood lymphocytes from healthy donors. Clastogenic activity was detected in concentrated ultrafiltrates of media from all six BS strains but none of the NF strains. The frequencies of chromosomal aberrations which were induced depended on the concentration of the ultrafiltrates. Addition of bovine Cu-superoxide dismutase to the blood lymphocyte cultures strongly suppressed the clastogenic potency of the ultrafiltrates. Unconcentrated conditioned BS media were inactive. From the pore-size of the ultra-filters and Sephadex G10 chromatography it is concluded that the clastogenic activity is in the molecular weight range of 1'000 to 10'000. The concentrated ultrafiltrates of BS culture media also possessed the capacity to induce sister chromatid exchanges in normal human blood lymphocytes but with relatively low efficiency. On the basis of these results and in analogy to certain collagen diseases such as Systemic Lupus Erythematosus we speculate that the primary genetic defect in BS may be a deficiency in the detoxification of active oxygen species.

DNA Repair, Cell Survival and Mutagenesis

551 MOLECULAR MECHANISM OF ERROR-PRONE DNA REPLICATION INDUCED IN UV-IRRADIATED OR ACETOXY-ACETYLAMINOFLUORENE TREATED MONKEY CELLS, Alain Sarasin, Claire Gaillard* and Annie Benoit, Institut de Recherches Scientifiques sur le Cancer, B.P. 8, F-94800 Villejuif and *Institut de Recherches en Biologie Moléculaire, Université Paris VII, Paris, France.
UV-irradiation or chemical carcinogen treatment of monkey kidney cells induces a new recovery pathway which enhances the survival of UV-irradiated simian virus 40 (SV40). We have demonstrated that in UV-irradiated cells, this phenomenon is indeed a very mutagenic process (1). We used phenotypic reversion to wild-type growth of two temperature-sensitive SV40 mutants (tsA58 and tsB201) as a measure of the rate of mutation. The DNA sequence analysis of one revertant genome (R-14-10) indicated that the back mutation is due to only one base-pair substitution in the middle of the T antigen gene. This substitution occurred opposite to a possible thymine dimer site and is located nine base-pairs apart from the original tsA mutation (the tsA58 mutation is due to one base-pair substitution : CG → TA).

tsA58 5'.....T-A-A-T-T-C-A-A...
R-14-10 5'.....T-A-A-A-T-C-A-A...

We also report here that this error-prone mode of DNA replication is induced in acetoxy-acetylaminofluorene-treated cells. As is the case after UV-irradiation, only UV-irradiated SV40 grown in treated cells give rise to a high mutation frequency in the progeny virus.

(1) Sarasin, A. and Benoit, A. Mutation Res., 1980, 70, 71-81.

552 THE USE OF AN HUMAN INDICATOR DNA SEQUENCE TO QUANTITATE GAMMA AND ULTRAVIOLET LIGHT RADIATION DAMAGE IN VITRO AND IN VIVO, William A. Haseltine, Judith Lippke, Lynn K. Gordon and Steven Grunberg, Sidney Farber Cancer Institute, Department of Pathology, Harvard Medical School, Boston, MA. 02115
The alpha sequence of human DNA is used as an indicator to measure the extent of radiation damage to DNA by gamma and ultraviolet light. The alpha sequence is a 342 base long, highly reiterated sequence comprising about 1% of the total cellular DNA. Use of this indicator sequence permits direct comparison of the locations of DNA damage produced by exposure of naked DNA or human cells to DNA damaging agents. Using this approach we have found that the effective dose of ultraviolet light seen by the alpha DNA in cells is about 50% that observed for similar exposure of naked DNA. Adjusting for this scaling factor, the distribution of ultraviolet light damage as a function of sequence is very similar. We have detected a novel cytosine photoproduct induced by ultraviolet light. This photoproduct results in alkali lability of the phosphodiester bonds. The lesion is neither a simple cytosine photohydrate nor a simple AP site. This lesion is stable in vivo and accounts for a major fraction of UV induced damage, even at low doses 20-50 J/m^2 doses. The lesion occurs exclusively at cytosines located 3' to pyrimidines. Similar experiments regarding the distribution of gamma ray damage to the human alpha sequence will also be reported.

553 ISOLATION OF SOMATIC MAMMALIAN CELLS DEFICIENT IN DNA REPAIR, Charles Waldren, Jeffrey N. Davidson, Andrew R. S. Collins, Eleanor Roosevelt Institute for Cancer Research, Denver, CO 80262
A new technique has been developed for isolating somatic mammalian cells deficient in mutagen-induced DNA repair processes. The method depends on the finding that breaks produced by repair endonuclease activity accumulate in the DNA of mutagen treated cells if the synthetic steps of excision repair are inhibited with hydroxyurea and 1-α-D-arabinofuranosylcytosine or by starvation of purine and pyrimidine-requiring auxotrophs for their required nutrients. The breaks facilitate unwinding and denaturation of DNA in alkali treated cells. Cells defective in repair endonuclease activity retain native DNA after treatment with alkali. Wild type and variant colonies are lysed in situ with alkali and their DNA is transferred (~95%) to nitrocellulose filters. The filter-bound DNA from repair-potent cells, being single stranded, is sensitive to S1 nuclease digestion. The DNA from colonies lacking endonuclease activity, being insensitive to S1 nuclease, remains on the filter, where, having been prelabeled by growth of the cells in ^{3}H-thymidine, it is detected by radioautography. Alternatively, filter-bound DNA may be located by annealing to it ^{32}P-labeled total DNA. Cell clones of interest are retrieved from a replica plate previously prepared from a master plate of the mutagenized colonies. This technique has been designed for the isolation of repair-deficient Chinese hamster ovary cells but appears to be generally applicable. Analysis of such mutants should help illuminate relationships between mutation, repair and carcinogenesis. (Contribution No. 334). Supported by grants (HD-02080), (CA-20810) and an ACS-Eleanor Roosevelt. Intn'l Cancer Fellowship from the Intn'l Union Against Cancer.

554 DEPENDENCE OF MUTATION FREQUENCY ON PLATEAU PHASE HOLDING TIME FOLLOWING UV IRRADIATION OF HUMAN DIPLOID FIBROBLASTS, Andrew J. Grosovsky and John B. Little, Harvard School of Public Health, Boston, Massachusetts 02115

We have investigated the induction of mutants resistant to 6-thioguanine (6-TG) following ultraviolet light exposure of density inhibited, plateau phase human diploid fibroblasts. The optimal post-treatment time for 6-TG selection and the maximum cell density permissible for complete recovery of all induced mutants have been determined. Dose response curves for cytotoxicity and mutagenicity are presented which quantify the effect of UV light exposure to plateau phase human diploid fibroblasts. In all of the above work plateau phase cultures were immediately replated at lower density following exposure.

Delaying subculture produced unexpected alterations in induced mutation frequencies. The irradiated cultures were sub-cultivated at various times from 0-24 hrs. following exposure. An increase in mutation frequency of approximately 3-fold was observed in cultures maintained in plateau phase for 3 hrs. This trend was reversed with longer holding times. The mutation frequency declined sharply in cultures held for 6 hrs. as compared to the 3 hr. value. Thereafter the mutation frequency showed a steady and gradual diminution.

These data indicate that the repair of potentially mutagenic damage is a complicated phenomenon which can lead to an increase or decrease in mutation frequency as a function of holding time. The decline in mutation frequency observed following longer holding intervals is consistent with the notion of potentially mutagenic damage repair being an error free process. However, the increased mutation frequency produced by a short holding period is consistent with the possibility of an error prone mode of repair in human diploid fibroblasts.

555 MUTATIONAL SPECIFICITY OF CARCINOGENS IN ESCHERICHIA COLI, Eric Eisenstadt, A. Jane Warren, David Atkins, Janis Porter, and Jeffrey Miller, Harvard University, Boston, MA and Universite de Geneve, Geneva, Switzerland.

We have determined a partial spectrum of mutational changes in DNA induced by 3,4-epoxycyclopenta(cd)pyrene, the biologically active metabolite of cyclopenta(cd)pyrene (CPP). CPP is a carcinogenic and mutagenic polycyclic aromatic hydrocarbon. The gene target was the lacI gene of Escherichia coli. Mutations in lacI were induced in an excision repair mutant of E. coli and in an isogenic strain carrying the mutation enhancing plasmid pKM101. Analyzing the distribution of lacI nonsense mutations induced by CPP-3,4-oxide revealed that 80% of all nonsense mutations at GC sites occurred via the GC to TA transversion event. Approximately 10% of all nonsense mutations arose via changes at AT sites. This underestimates the incidence of base pair substitutions at AT sites since nonsense mutations are not generated by AT to GC transitions. Plasmid pKM101 enhanced the overall incidence of mutations by 10-fold but did not change the relative frequency of specific base pair substitutions. We found that approximately 10% of all lacI mutations induced by CPP-3,4-oxide were nonsense. Since the nonsense mutations detectable in this study represented only one-sixth of the lacI codons, and since only certain base pair substitutions in these codons generate nonsense mutations, base pair substitutions must be a common mutational event following treatment with CPP-3,4-oxide. We have confirmed this more directly by demonstrating that 38 of 40 non-suppressible mutants in the amino-terminal portion of lacI show negative complementation. We are now characterizing lacI mutations induced by benzo(a)pyrene diol-epoxide.

556 REGULATION AND FUNCTION OF ESCHERICHIA COLI GENES INDUCED BY DNA DAMAGE, Graham C. Walker, Anne Bagg, Stephen J. Elledge, and Cynthia J. Kenyon.

We have identified a set of six unlinked din (damage-inducible) genes in E. coli. These genes were originally detected as din-lac operon fusions by screening for mitomycin-C-inducible β-galactosidase synthesis in cells carrying random insertions of the Mud(Ap, lac) operon fusion vector. The expression of β-galactosidase in these fusion strains is increased by a number of agents or conditions which induce the "SOS" responses. The induction of din genes is coordinately controlled by the recA$^+$lexA$^+$ regulatory circuitry. The protease activity of the recA protein is required for induction, since no induction is seen in a background containing a particular recA mutation which eliminates the protease activity of recA protein but not its recombination functions. Analysis of the effect of lexA, spr, and spr recA mutations on the expression of din-lac fusions leads us to conclude that the lexA protein directly represses each din gene. Thus, increased expression of these din genes appears to result directly from the recA-catalyzed cleavage of the lexA protein at the time of "SOS" induction. By isolating and characterizing an additional set of uvrA::Mud(Ap, lac) fusions we have conclusively demonstrated that one of these damage-inducible genes is uvrA. In addition we have obtained an insertion of Mud(Ap, lac) into umuC, a gene whose product is required for most chemical mutagenesis. β-galactosidase synthesis in this fusion strain is strongly stimulated by UV in a recA$^+$lexA$^+$-dependent fashion. We are currently cloning this gene.

557 CELL SURVIVAL, PROPHAGE INDUCTION AND MUTAGENESIS INDUCED BY 8-METHOXYPSORALEN PLUS ULTRAVIOLET LIGHT-A IN THE AMES MUTAGEN TESTER STRAINS, L. WHEELER, M. DeMeo, B. Hirsch and G. Smetana, Wadsworth VA Hospital and UCLA, Los Angeles, CA 90024

8-methoxypsoralen (8-MOP) is not detected as a mutagen in the Ames Test either in the presence or absence of S9-mix and/or ultraviolet light-A(320-400nm). Wheeler et al (Proc. W. Pharm. Soc. 22:261, 1980) has recently shown that Ames strains harbor prophages that are inducible by carcinogens and mutagens. 8-MOP plus UVA (PUVA) was found to be a potent prophage inducing treatment. Significant induction was observed in TA1535, TA1538 and TA100 with 50 ng/ml and 0.25 J/cm^2 of UVA. PUVA is also cytotoxic to the mutagen tester strains:

	SURVIVAL (N/N_o)*				
Strain	0.125(ug/ml 8-MOP)	0.25	0.50	1.0	2.0
TA1535	0.91	0.40	0.28	0.13	0.014
TA100	0.31	0.17	0.03	0.003	0.0005
TA1538	0.56	0.07	0.006	0.001	0.00007

*cells were treated with 0.25 J/cm^2 UVA

The frameshift (FS) tester strain TA1538 is more sensitive to the bactericidal effects of PUVA than the base pair (BP) tester strains TA1535 and TA100. This sensitivity cannot be explained by increase prophage induction in TA1538. One of several explanations studied were the differences in lysogeny between FS and BP strains. In BP strains two phages (Fels 1 and Fels 2) are detectable while only Fels 1 can be detected in FS strains. In conclusion the data suggest that PUVA is unique in that plasmid pKM101 does not confer enhanced survival to TA100 compared to TA1535 in contrast to many other carcinogens and mutagens.

558 A ROLE FOR SINGLE-STRANDED DNA-BINDING PROTEIN IN THE CONTROL OF DNA REPAIR, Ralph R. Meyer, Diane C. Rein and John M. Trela, University of Cincinnati, Cincinnati, OH 45221

Prokaryotic systems have proved to be useful models for understanding basic mechanisms of DNA repair. One system receiving considerable attention in recent years is the inducible, error-prone repair system ("SOS repair") of E. coli. This pathway is mediated through induction of recA protein by agents which damage DNA or interrupt DNA replication. Current models for the regulation of this pathway involve repression by a protein coded by the lexA gene. DNA damage leads to activation of recA protein, present at low constituitive levels, such that it acts as a protease and cleaves the lexA repressor. This results in induction of high levels of recA needed for postreplicational repair. Our interest in this system stems from our finding that mutants in single-stranded DNA-binding protein (ssb mutants) are UV-sensitive and appear to be defective in recA-mediated repair. We have found that ssb mutants fail to induce normal levels of recA protein after nalidixic acid or mitomycin C treatment. Moreover, strains carrying the cloned wild-type ssb$^+$ gene overproduce binding-protein 10 to 15-fold. These cells are also UV-sensitive and fail to induce normal levels of recA protein. This suggests that single-stranded DNA-binding protein may play a role in regulating SOS repair by acting as a corepressor for the recA gene.

559 RADIATION-SENSITIVE BHK CELLS: ISOLATION AND PRELIMINARY CHARACTERIZATION, Helen H. Evans, Karen G. Glazier, Thomas E. Evans, Matthew C. Weber, and Min-Fin Horng, Case Western Reserve University, Cleveland, Ohio 44106.

The host-cell viral reactivation suicide enrichment procedure for DNA repair-deficient mutants, first described by Howard-Flanders and Theriot (Genetics 47, 1219, 1962) and subsequently used successfully in eukaryotic cells by Shiomi and Sato (Somatic Cell Genetics 5, 193, 1979) was utilized to enrich for radiation-sensitive strains in a population of mutagenized BHK cells. Nine surviving colonies were obtained after two rounds of infection of these cells with heavily irradiated Herpes simplex virus (HSV). (The dose to HSV was 109 krad, which reduced the plaque forming units to 14% of the original value.) Three of the nine isolates appear to be sensitive to ionizing radiation. One of the radiation-sensitive strains (V_2) showed a 50% reduction in the reactivation of γ-irradiated HSV. Semi-quantitative screening using a microtiter dish method has indicated that strain V_2 is also sensitive to methylmethane sulfonate, ethylmethane sulfonate, and 4-nitroquinoline-1-oxide. Five of the isolates displayed a sensitivity to N-methyl N'-nitro N-nitrosoguanidine which was similar to the sensitivity of wild-type BHK cells. Further tests of the response of these strains to alkylating agents are in progress. The investigation of the response of these strains to the mutagenic effects of ionizing radiation and alkylating agents is planned.

(Supported by NIH grant CA-23427 and DOE Contract DE-AC02-77EV04472)

560 MUTATIONAL SPECIFICITY OF ALKYLATING AGENTS: INFLUENCE OF CELLULAR REPAIR MECHANISMS. Barry W. Glickman, Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709 and J. Brouwer, Laboratory for Molecular Genetics, Rijksuniversiteit te Leiden, The Netherlands.
The influence of dose and cellular repair capacity on the mutagenic specificity of methylmethane sulphonate (MMS) and ethylmethane sulphonate (EMS) has been studied using the lacI forward mutational system. The lacI system allows the determination of the precise nature of base substitutions at 65 sites in the lacI gene of Escherichia coli. In the wild-type strain EMS produces largely G:C to A:T transitions, however, excision repair deficient UvrB⁻ strains are hypermutable by EMS and both transitions and transversions are produced. A Dam⁻ strain, deficient in methylation instructed mismatch repair, also produced both transition and transversion mutations following treatment with EMS. In contrast, the hypermutable Alk⁻ strain, defective in the repair of alkylation damage, did not result in an altered EMS mutational spectrum. Treatment of the wild-type strain by MMS resulted in the production of both transitions and transversions. This mutagenesis was totally recA⁺- dam⁺ dependent. We are presently investigating the role of error-prone repair in determining the mutational specificity of MMS induced mutagenesis by analyzing the mutational spectrum in a strain carrying a mutation in the umuC gene which is essential for the expression of error prone repair. Data on mutational specificity of these agents in a wide variety of repair deficient mutants at a broad range of doses will provide information on what target lesions are important for mutagenesis and which DNA repair pathways are involved in determining the ultimate fate of these lesions.

561 INVOLVEMENT OF DNA REPAIR GENES IN SPONTANEOUS MUTAGENESIS OF Escherichia coli: IMPLICATIONS FOR SPONTANEOUS CARCINOGENESIS, Neil J. Sargentini and Kendric C. Smith, Stanford University School of Medicine, Stanford, CA 94305
Spontaneous mutation rates were determined for E. coli strains deficient in DNA repair. Mutation assays scored UAG, UAA and frameshift reversion, and mutations to valine resistance. Excision repair-deficient strains (uvrA6, uvrA155, uvrB5, and ΔuvrB) exhibited two to thirteenfold higher spontaneous mutation rates (depending on the mutation scored) than were found for isogenic wild-type strains. The uvrA strains were not significantly different from the uvrB strains. This enhanced spontaneous mutagenesis was absent in uvrB5 strains that also carried lexA101, umuC36 or both uvrD3 and recB21 mutations. We also found the spontaneous mutation rates for uvr⁺ strains carrying recA56 or lexA101 mutations to be significantly lower than those for isogenic wild-type strains. Thus, it appears that the DNA repair genes that control UV radiation mutagenesis control most of spontaneous mutagenesis. We conclude that spontaneous lesions, that can be dealt with by uvrA uvrB-dependent excision repair, arise in the DNA of E. coli and that these lesions when not excised give rise to mutations via recA lexA-dependent error-prone repair, in wild-type cells as well as in uvrA and uvrB strains. This excisable DNA damage, that results from normal metabolism, gives rise to spontaneous mutations in bacteria and could be the molecular basis of spontaneous carcinogenesis in humans as suggested by Totter (PNAS 77:1763, 1980). (Research supported by Public Health Service research grant CA-02896 and research program grant CA-10372 from the National Cancer Institute.)

562 MUTATIONS INDUCED IN SYNCHRONOUS CHO CLONES BY CARCINOGENS-MUTAGENS. H. John Burki, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720.
When CHO cells are synchronized by mitotic selection and treated with carcinogens-mutagens like X rays, UV and ENU at different times in the cell division cycle, characteristic "age responses" for the induction of clones resistant to 6-thioguanine, diphtheria toxin, and ouabain occur. The mutagenic sensitive periods are in the "G1" period, or the "S" period and are characteristic of the mutagenic endpoint and the carcinogen-mutagen used. These synchronous results suggest that differential biochemical events during the cell cycle are important in the processes leading to the production of mutations in "wild type" CHO cells. CHO clones sensitive to the mutagenic effects of carcinogens-mutagens have been isolated using a modified replica plating technique. These clones are similar to wild type clones in sensitivity to X rays but are ultrasensitive to UV radiation. Clone 27-1 and clone 43-3B are hypersensitive to UV induced resistance to 6 thioguanine, ouabain and diphtheria toxin. The variation in sensitivity in the cell cycle which is so characteristic of wild type cells was greatly reduced both for induced reproductive death and mutation.

These results strongly suggest that an efficient "error free" repair process exists in "wild type" CHO cells. Absence of characteristic "age response" in carcinogen-mutagen sensitive CHO mutants suggests that the successful operation of this "error-free" repair process is dependent on biological events during the cell life cycle.

563 ISOLATION AND PARTIAL CHARACTERIZATION OF MUTANTS OF SACCHAROMYCES CEREVISIAE ALTERED IN SENSITIVITIES TO LETHAL EFFECTS OF BLEOMYCINS, Carol W. Moore, University of Rochester, Rochester, NY 14642

Two of eight mutants (bmr) isolated in Saccharomyces cerevisiae on the basis of their increased resistance to lethal effects of antitumor bleomycins (BM), and about two-thirds of 180 yeast mutants (bms) isolated on the basis of their increased sensitivities to cell-killing by phleomycins (PM) or BM were sensitive to one or more of the agents UV, X-rays or hydrogen peroxide. Thus, these mutants are likely to be altered in processes acting directly or indirectly on DNA damage. The range of increased sensitivities to each agent was two to eightyfold over the parental strain, with increases of two- to fourfold being the most common over the dose ranges tested. Of particular interest is the identification of a new category of yeast mutants which are PM- (bms) and UV-sensitive, but not X-ray sensitive. None of the radiation-sensitive (rad) mutants of yeast, isolated in other laboratories on the basis of increased sensitivities to killing by UV or X-rays, possessed this phenotype (C. Moore, 1978, Mutat. Res. 51:165-180; submitted for publication).

The remaining six bmr mutants and approximately 60 bms mutants appear as resistant as the parent strain to cell-killing by UV or X-rays, and are likely therefore, to be altered in cell wall or membrane function.

A genetic basis for the phenotypes of several of the bmr and bms mutants has been established.

564 REPAIR OF 3-METHYLADENINE AND 7-METHYLGUANINE IN NUCLEAR DNA OF CHLAMYDOMONAS: REQUIREMENT FOR PROTEIN SYNTHESIS, Gary D. Small and James M. Sweet, Section on Biochemistry, Division of Biochemistry, Physiology and Pharmacology, The University of South Dakota, Vermillion, South Dakota, 57069.

The removal of 3-methyladenine and 7-methylguanine from nuclear DNA was determined following exposure of *Chlamydomonas reinhardi* to methyl methanesulfonate (MMS). The amount of 3-methyladenine in DNA was determined using an extract from *Micrococcus luteus* that has a 3-methyladenine-DNA glycosylase. The amount of 7-methylguanine was estimated by heating the DNA for 30 min at 70° followed by alkaline hydrolysis of the resulting AP sites. The molecular weight of the DNA was determined using alkaline sucrose gradients. The 3-methyladenine is removed with a half-life of 2-3 hrs whereas the 7-methylguanine is removed with a half-life of 10-12 hrs. The rate of removal of the 7-methylguanine is more than an order of magnitude faster than the estimated non-enzymatic hydrolysis rate indicating the probability of an enzymatic repair.

Addition of cycloheximide immediately after MMS treatment inhibits the removal of 3-methyladenine and 7-methylguanine from DNA. If cycloheximide is added 1 1/2 hrs after treatment with MMS, there is much less inhibition of the removal of 3-methyladenine. These results are interpreted to mean that MMS induces the synthesis of one or more proteins that are required for the repair of 3-methyladenine from *Chlamydomonas* DNA. (Supported by USPHS grant GM21095).

565 ULTRAVIOLET LIGHT-INDUCED RECOVERY IN ESCHERICHIA COLI OF THYMINE RING SATURATION IN ØX174-DNA, Phillip M. Achey and Cynthia A. Femia, Univ. of Florida, Gainesville, 32611

Induced recovery of 5,6-dihydroxy-dihydrothymine (t') damage in DNA was investigated. Treatment of single-strand, circular ØX174-DNA with osmium tetroxide, which introduces predominately t' damage in DNA, causes loss in biologically infectivity of the DNA in spheroplasts of E. coli. Approximately 62% of the biological damage resulting from oxidation of ØX174-DNA by osmium tetroxide is repairable by the induced recovery system of E. coli. It is significant that no enhanced recovery was observed when treated DNA was assayed in induced cells of E. coli AB 2463 recA, which is deficient for induced repair, thus indicating that the enhanced survival observed in this study is dependent on a proficient induced repair system. Quantitatively, the efficiency of induced repair of DNA oxidized by osmium tetroxide is higher than for any of the conditions of gamma irradiation previously reported from this laboratory, indicating that t' base damage is more susceptible to biological recovery than the conglomerate of biological damage from gamma-rays.

566 MUTAGENESIS AND REPAIR OF ACETOXYACETYLAMINOFLUORENE-DNA ADDUCTS, William D. Taylor, Karen R. Cover, Cindy Luisi and Ronald D. Porter, Pennsylvania State University, University Park, Pa. 16802

The ratio of C-8 to N-2 adducts formed in PBR 322 DNA and recombinants between ØX174 DNA and PBR 322 DNA by reaction with acetoxyacetylaminofluorene was varied by modifying the conditions of reaction. H^3 labeled carcinogens and C^{14} labeled DNA have been used to quantitate the number of adducts. Enzymatic hydrolysis of DNA followed by LH-20 chromatography and HPLC have been used to measure the adduct ratios. The effect of these variations on DNA repair was observed by transfection of a series of repair deficient E. coli mutants. C-8 adducts are repaired by excision repair; N-2 adducts block DNA replication. Forward mutations in a number of ØX174 and PBR322 genes have been used to determine the role of these adducts in mutagenesis.

567 REDUCED ABILITY OF REPAIR-DEFICIENT MUTANTS OF CHO CELLS TO REPAIR DNA LESIONS INDUCED BY 4-NITROQUINOLINE-1-OXIDE, Raymond E. Meyn, Susan F. Jenkins, Sarah E. Locher, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas.

Much of the current understanding of relationships between DNA damage, DNA repair, and cellular endpoints such as survival and mutation has come through the use of DNA repair-deficient mutants. Research with mammalian cells has primarily taken advantage of the availability of human cells derived from diseases such as Xeroderma pigmentosum and Fanconi's anemia. However, the recent isolation of a variety of repair-deficient mutants in an established cell line (CHO) by Dr. Larry A. Thompson at the Lawrence Livermore Laboratory, Livermore California, offers several advantages for these types of investigations. We have characterized two of these mutants (UV-20 and UV-5) which have been shown by Thompson and co-workers to be sensitive to ultraviolet light (UV) and the potent carcinogen 4-Nitroquinoline-1-oxide (4NQO) for their ability to repair 4NQO lesions in their DNA. These lesions were detected as alkaline labile sites in DNA by the sensitive technique of alkaline elution. The same level of sites was present to both mutant and wild-type cells immediately after treatment, but after 24 hr. of incubation the wild-type cells had removed nearly all of the sites while a substantial number of sites remained in both mutant cell lines. These findings indicated that repair of DNA lesions induced by the carcinogen 4NQO is identical or at least has some steps in common with the pathway responsible for excision of UV-damage in CHO cells. (Supported by NIH/NCI Grants CA-23270 and CA-26312).

568 AN INDUCIBLE DNA-BINDING PROTEIN IN MAMMALIAN CELLS, Udo Mallick, Hans-Jobst Rahmsdorf, Helmut Ponta & Peter Herrlich, Kernforschungszentrum Karlsruhe, Institut für Genetik, D-7500 Karlsruhe 1, Fed. Rep. Germany

Treatment of permanent mammalian cell lines and of primary cells with agents that inhibit DNA replication, causes the induction of a family of proteins. The pattern of induced proteins varies with species and with cell type. The major products in fibroplasts and in lymphoid cells migrate to similar positions in twodimensional polyacrylamide gels. They differ, however, in the peptide pattern generated by limited proteolysis in SDS.

The most prominent induced protein in lymphoid cells has been examined further. We give it the preliminary designation protein XM1 (M for mammalian). While in several myeloma cell lines protein XM1 is induced by agents such as mitomycin C hydroxyurea or by Gamma irradiation, the very same protein is synthesized at constitutive rate in resting or polyclonally activated B (and T) spleen lymphocytes. The induction is prevented by actinomycin D suggesting regulation on the transcriptional level. Protein XM1 is located in the cell nucleus and it binds to DNA. The latter property has been used for purification.

569 Fidelity of DNA Synthesis In Vitro by Carcinogen-Reacted DNA Polymerases and Carcinogen modified Templates, John Y.H. Chan and Frederick F. Becker, M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030 (Supported by N.I.H. Grant CA 20657) Decreased fidelity in DNA replication and repair has been proposed as a mechanism for chemical carcinogenesis. To elucidate the underlying mechanism of abnormal fidelity, the direct acting carcinogens, methylnitrosurea (MNU) and N-acetoxy-N-2-acetylaminofluorene (AcAAF), were studied after interaction with either DNA polymerases or the appropriate templates. Purified DNA polymerases α and β from rat liver were reacted with AcAAF or MNU, and the fidelity of DNA synthesis was assayed in vitro by measuring the incorporation of noncomplementary nucleotides into synthetic copolymers. DNA polymerase α was extremely sensitive to inactivation by both carcinogens while polymerase β was relatively resistant. However, no decrease in fidelity was observed for either enzyme. These results also support our previous in vivo and in vitro data that DNA polymerase β, a putative component of the normal repair machinery, is resistant to carcinogen damage. When the fidelity assays were repeated using normal enzymes, but with carcinogen-modified poly(dA-dT) or poly(dC-dG), decreased fidelity was observed with the MNU-reacted template but not the AcAAF-reacted template. These data are in accord with the concept that MNU results in small methylated adducts, which allow slippage and misincorporation to occur; while AcAAF-adducts, which are large, result mainly in the termination of synthesis. Since neither AcAAF-modified templates nor AcAAF-reacted enzymes could be shown to decrease fidelity of DNA synthesis in vitro, a different mechanism is probably responsible for AcAAF induction of neoplasia, or some other factor(s) may be required for a decrease in fidelity such as the aberrant DNA-polymerase α which has been identified. (Supported by NIH Grant CA 20657)

570 INDUCTION OF MUTATIONS IN CHO CELLS AFTER CHRONIC PRETREATMENT WITH MNNG, Jeffrey L. Schwartz and Leona Samson, Laboratory of Radiobiology, University of California, San Francisco, CA. 94143

Chronic pretreatment of a Chinese hamster ovary (CHO) cell line with non-toxic levels of N-methyl-N'-nitro-nitrosoguanidine (MNNG) renders these cells highly resistant to the cytotoxic effects of subsequent MNNG challenges and slightly resistant to the induction of sister chromatid exchanges (SCEs). There is no change in the induced frequency of 6-thioguanine-resistant mutants per survivor when cells are challenged with MNNG doses that range from 0.05 to 0.5 μg/ml. At very high challenges, however, mutation frequency declines such that after 1.0 μg/ml MNNG, the mutation frequency of pretreated cells is 5-fold lower than controls. Previous studies had suggested that the resistance to the cytotoxic and SCE-inducing effects of alkylating agents was probably achieved by the cells becoming more efficient at repairing alkylation damage, analogous to the adaptive response in E. coli. The pretreatment dose is crucial, however, and thus the failure to detect any reduction in induced mutation frequency at low challenge doses, may reflect a less than optimal pretreatment regime. Alternatively, it may be due to the reported inability of CHOs to repair the premutagenic lesion, O-6-alkylguanine. The reduced mutation frequency at high MNNG concentrations probably involves a completely separate process. Studies designed to answer these questions are now in progress. Work supported under the auspices of the U.S. Department of Energy.

571 INDIRECT INDUCTION OF MUTAGENESIS OF INTACT PARVOVIRUS H-1 IN MAMMALIAN CELLS, J. Rommelaere, J.J. Cornelis, Z.Z. Su, and D.C. Ward, Dept. of Molecular Biology, Universite Libre de Bruxelles, 1640 Rhode St Genese, Belgium, and Dept of Human Genetics, Yale University School of Medicine, New Haven, Ct. 06510, U.S.A.

Mammalian cells from rat and human origin display a mutator activity towards intact parvovirus H-1 if they have been exposed to ultraviolet light or to chemical mutagens of the 2 naphtofuranne series prior to infection. Enhanced mutagenesis of intact H-1 could be induced to almost the same extent in untreated cells by infecting them with homologous or heterologous virus which had been irradiated with ultraviolet light. Mutagenesis was assayed by determining the reversion rates of a thermosensitive late H-1 mutant to wild-type phenotypes at the restrictive temperature. The cellular process leading to the generation of a higher frequency of mutants among the descendants of unirradiated H-1 requires de novo protein synthesis shortly after cell treatment, suggesting that it might be inducible.

572 IN VITRO HOST-CELL REACTIVATION OF ALKYLATED T7 DNA, Lori A. Dodson* and Warren E. Masker, University of Tennessee—Oak Ridge Graduate School of Biomedical Sciences and Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830

We have investigated the effect of alkylating agents on survival of bacteriophage T7 and have characterized in vitro DNA synthesis and in vitro packaging of T7 DNA exposed to MMS or EMS. Survival of intact T7 phage treated with different concentrations of MMS or EMS was dramatically reduced compared to wildtype when titered on a strain deficient in 3-methyladenine-DNA glycosylase (tag); intermediate survival was observed for a polA host. Purified T7 DNA was similarly treated and subsequently packaged in vitro to form viable phage. This in vitro system closely mimics the in vivo situation with respect to chemical DNA damage when biological activity of packaged DNA was used as the endpoint. On a molar basis, treatment of intact phage or purified DNA with MMS was more toxic than similar treatment with EMS. Analogous to the in vivo situation, survival was lowest when the tag mutant was used both to prepare the packaging extract and as the indicator strain. Furthermore, during the packaging reaction we have observed in vitro repair of DNA treated with different concentrations of MMS or EMS. When titered on a tag mutant, survival of phage resulting from packaging of damaged DNA by an extract from a tag mutant could be enhanced by complementation with an extract from an uninfected tag^+ strain, or alternatively, by packaging with an extract from wild-type cells. (*Predoctoral fellow supported by NIH Grant GM 7438. Research sponsored by the Office of Health and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with the Union Carbide Corporation.)

573 SELECTION OF MITOMYCIN C RESISTANT S49 MOUSE T-LYMPHOMA CELLS. Lorraine J. Gudas and Michael Roguska, Pharmacology Dept., Harvard Medical School, and Sidney Farber Cancer Institute, Boston, MA 02115.

We have mutagenized S49 mouse T-lymphoma cells using N-methyl-N'-nitro-nitrosoguanidine. After growth for several generations to allow expression of the mutations, we plated the cells in 0.32% agarose over a mouse embryo fibroblast feeder layer in the presence of 0.05 μg/ml mitomycin C. Mutant cells resistant to mitomycin C were obtained at a frequency of approximately 2×10^{-6}. One of these mitomycin C resistant mutants, MC-2-2, is three to four-fold more resistant than its wild-type S49 parent to the drug. The mutant MC-2-2 is not cross-resistant to N-methyl-N'-nitro-nitrosoguanidine or bleomycin. When the sensitivity of this mutant MC-2-2 to deoxyribonucleosides was measured, we found that the cell line MC-2-2 was not more sensitive than the wild type parent to deoxyadenosine (in the presence of the adenosine deaminase inhibitor EHNA), but that the mutant MC-2-2 was greater than two-fold more sensitive to thymidine when compared to its parent. Moreover, the deoxyribonucleotide pools were altered in the mutant MC-2-2, as measured by high pressure liquid chromatography. Further study of this and other mutants should help delinate the relationship between the levels of deoxyribonucleotides and mutagenesis in cells.

574 CRITICAL TEMPERATURE AND LEVEL OF DAMAGE FOR MMS INDUCED STRAND BREAK REJOINING IN CHICK EMBRYO FIBROBLAST DNA, Burt V. Bronk and Joe D. Patton, Clemson University, Clemson, South Carolina 29631.

Mammalian cells have repair systems (for DNA damage following various treatments) which are inhibited at temperatures higher than about 40.5°C but which apparently function at temeratures between 37°C, the normal mammalian body temperature and 40.5°C, above which cell growth is also inhibited. We have found that chick cells grow about equally well and with similar doubling times at 37°C or at 42°C, a normal deep body temperature for chickens. We assessed repair by disappearance of the alkali sensitive bonds which appear in DNA as part of the repair process following damage by the alkylating agent methyl methane sulfonate (MMS). We followed repair at various incubation temperatures between 37° and 43° for several hours after damage at a particular level measured immediately after MMS treatment. From the mammalian results, one might expect repair to occur at temperatures several degrees above 42°C. However we found that repair was completely inhibited at temperatures of 43°C or higher. At all other temperatures we found a critical level of damage somewhat lower than about 0.6 breaks per control length of roughly 0.9×10^8 daltons (number average molecular weight). This critical level did not change appreciably for post-treatment incubation temperatures between 37°C and 42.5°C.

575 TWO 3-METHYLADENINE DNA GLYCOSYLASES AND A 7-METHYLGUANINE DNA GLYCOSYLASE FROM E. COLI, David A. Goldthwait, Lorette K. Thomas and Chul-Hak Yang, Case Western Reserve University, Cleveland, OH 44106

Two different 3-methyladenine DNA glycosylases have been isolated from E. coli and designated 3-methyladenine DNA glycosylase I (I) and 3-methyladenine DNA glycosylase II (II). I has been described by Riazzudin and Lindahl (Biochemistry 17, 2210, 1978). The two activities can be separated on DEAE, or phosphocellulose columns or by isoelectric focusing. The molecular weight of I is 20,000 and of II is 27,000. I releases 3-methyladenine and 3-methylguanine while II releases 3-methyladenine, 3-methylguanine, 7-methyladenine and 7-methylguanine. The apparent K_m for 3-methyladenine of I is 13.5 x 10^{-9} and of II is 9.2 x 10^{-9} M. Only I is inhibited by 3-methyladenine with a K_i of 2.6 x 10^{-3} M. At 2.5 and 5.0 mM 3-methyladenine, the inhibition is uncompetitive, while at 7.5 mM it is more nearly non-competitive. Both enzymes are active in 1 mM EDTA; I is stimulated 23% by 5 mM Mg^{++} which has no effect on II. Both enzymes have a broad pH optimum between 7 and 9 and both are free of exonuclease, double strand, single strand, and apurinic endonucleases. Mutants tag 1 and tag 2, provided by Dr. Lindahl are both lacking I. Dr. Lindahl has also observed the second 3-meA activity. The activity for 7-methylguanine to date has not been separated from that for 3-methyladenine by chromatography on various columns or by isoelectric focusing. The relative rates of release of 3-methyladenine to 7-methylguanine by II are 30 to 1.

576 ENHANCED MUTAGENESIS DUE TO DEPURINATION, Thomas A. Kunkel, Roeland M. Schaaper, Clyde W. Shearman and Lawrence A. Loeb, Gottstein Memorial Cancer Research Laboratory, University of Washington, Seattle, WA 98195.

Depurination, the release of purine bases from DNA due to the breakage of the N-glycosylic bond, is a frequent cellular event. The rate constant of depurination in vitro suggests that as many as 20,000 stable apurinic sites may be formed per day in a mammalian cell. Furthermore, carcinogen treatment may enhance the rate of depurination as much as 100- to 1000-fold. We have assessed the mutagenic consequences of depurination in vitro using three methods. Firstly, depurination leads to an increased mis-incorporation of non-complementary nucleotides into synthetic polynucleotides when copied by purified DNA polymerases. Secondly, depurination of ϕX174 am3 DNA in vitro leads to enhanced mutagenesis when this DNA is copied by Pol I. Alkaline and neutral sucrose gradient analysis of a single restriction endonuclease fragment obtained from the product of the Pol I reaction on depurinated ϕX174 DNA clearly indicates that Pol I can copy past apurinic sites, albeit at a reduced rate. Thirdly, depurination of ϕX174 am3 DNA is highly mutagenic when transfected (without in vitro synthesis) into spheroplasts derived from bacteria which were previously exposed to UV light to induce SOS repair. The increase is as great as 20-fold and is proportional to the number of apurinic sites introduced into the DNA. The mutagenic effects of depurination in all three assays can be reversed by alkali treatment of the depurinated DNA, suggesting that it is indeed the depurinated site that is responsible for the mutagenic effect. These data suggest the possibility that at least some of the mutagenesis associated with known carcinogens results from mis-incorporation opposite apurinic sites.

577 HOST-CELL REACTIVATION OF HERPES SIMPLEX VIRUS TYPE 1 IN TRANSFORMED AND NORMAL BHK CELLS, Karen G. Glazier, Matthew C. Weber and Helen H. Evans, Case Western Reserve University, Cleveland, OH 44106

The DNA-repair capabilities of BHK cells were investigated by measuring the reactivation of irradiated HSV1 as compared to its reactivation by normal and repair-deficient human diploid fibroblasts. BHK cells were found to have an intermediate ability to reactivate UV-irradiated HSV1 relative to normal human fibroblasts and xeroderma pigmentosum (XP) group A cells. In contrast, BHK and normal human cells were equally able to reactivate HSV1 treated with ionizing radiation; whereas, xeroderma pigmentosum cells were slightly less efficient in the repair of γ-irradiated virus. Ultraviolet enhanced reactivation (UVER) of UV-irradiated HSV1 was demonstrated immediately as well as up to 48 hours after irradiation of BHK monolayers. The maximal UVER (approximately 3 fold) was seen when the BHK cells were irradiated 24 hours prior to infection with virus. Host-cell reactivation (HCR) and induced HCR are also being investigated in transformed BHK cells. Preliminary results show that transformed BHK cells are equivalent to the nontransformed BHK cells with respect to HCR but demonstrate little or no UVER at 24 hours after irradiation of the transformed monolayers. (Supported by NIH grant CA-23427 and DOE contract DE-AC02-77EV04472.)

578 NORMAL CHROMOSOME BREAKAGE, SISTER CHROMATID EXCHANGE AND UNSCHEDULED DNA SYNTHESIS OBSERVED IN HEREDITARY RETINOBLASTOMA, Jill D. Fabricant, William Au[+] Robert N. Fabricant*, Andrew F. Frost and Keith Morgan*, University of Texas Medical Branch, Galveston, TX 77550, [+]M. D. Anderson, Houston, TX 77030, and * Louisiana State University Eye Center, New Orleans, LA 70112

It has been suggested that cells from patients with hereditary retinoblastoma are more sensitive to the lethal effects of x-rays than are cells from normal controls (Weichselbaun et al., 1978). From this work, as well as that from the high incidence of second tumors in these patients, a defect in DNA repair has been postulated. In this study, we analyzed spontaneous chromosome breakage, spontaneous and mitomycin C-induced sister chromatid exchange (SCE) frequency as well as unscheduled DNA repair (UDS) by mitomycin C and MNNG in two patients (father and daughter) with hereditary retinoblastoma and in three normal controls. An increase was not observed in spontaneous breakage, nor were differences observed in the SCE frequencies (both spontaneous and induced) in the patients when compared to controls. Lymphocytes were studied for UDS and all similarly treated cells had comparable values. Our data, therefore, suggest that DNA repair, at least as measured by SCE and UDS occurs normally in some patients with hereditary retinoblastoma.

579 THE ROLE OF DEOXYNUCLEOSIDE TRIPHOSPHATE POOLS IN THE CYTOTOXIC AND MUTAGENIC EFFECTS OF DNA ALKYLATING AGENTS, Mark Meuth, Clinical Research Institute of Montreal, Montreal, Quebec H2W 1R7, Canada.

The objective of these studies was to define the role of deoxynucleoside triphosphate pools in the cytotoxic and mutagenic effects of DNA alkylating agents. Survival of Chinese hamster ovary (CHO) cells after treatment with DNA alkylating agents was clearly related to the balance of the dCTP and dTTP pools -- high dCTP/dTTP ratios increased the survival of CHO cells 2- to 10-fold compared to treatment in low dCTP/dTTP. Plotting the toxicity of one agent, ethylmethane sulfonate (EMS) against the dCTP/dTTP pool content of our CHO strains gave "kinetics" consistant with a competition between dCTP and dTTP determining the cytotoxicity of EMS. Induction of mutations at three genetic loci by EMS was also affected by pool alterations. Although the maximum mutagenesis obtained in high or low dCTP/dTTP was not significantly different, it took considerably lower concentrations of EtMes to obtain this maximum in conditions giving low dCTP/dTTP. Currently we are attempting to correlate the mutational events induced by EMS at specific genetic loci with dCTP/dTTP pool content. The deoxynucleoside triphosphate pools clearly affect an event subsequent to DNA alkylation as shifting cultures from high dCTP/dTTP to low *after* EMS treatment gave nearly identical survival and mutagenesis as cultures with a constantly low dCTP/dTTP. We are also investigating the cytotoxic and mutagenic effects of EMS on CHO mutant strains having altered levels of dCTP/dTTP. These results are consistent with a common mechanism: mispairing of thymidine with the O^6 alkylated guanine -- causing both the cytotoxic and mutagenic effects of EtMes.

580 1,N^6-ETHENOADENOSINE AND 3,N^4-ETHENOCYTIDINE IN POLYRIBONUCLEOTIDES LEAD TO TRANSCRIPTIONAL ERRORS AND AMBIGUITY, *Sylvia J. Spengler and B.A. Singer, University of California, Berkeley, CA 94720.*

The human carcinogen, vinyl chloride, is metabolically converted by the microsomal cytochrome P-450-dependent monooxygenases to the reactive compound chloroethylene oxide, which also rearranges to form chloroacetaldehyde. These compounds react with adenosine and cytidine to form the fluorescent etheno derivatives, 1,N^6-ethenoadenosine (εA) and 3,N^4-ethenocytidine (εC). We have synthesized C and A ribopolymers containing varying amounts of εC or εA and transcribed them using DNA-dependent RNA polymerase in the presence of Mn^{2+}. Nearest neighbor analysis of the transcripts showed that εA acted more like U than like A and more like A than G. Etheno C, on the other hand, acts primarily like A, though simulation of U and G also occurred. Neither derivative behaved like C in these copolymers. Such transcriptional errors may indicate the nature of the mutagenic lesion involved in vinyl chloride carcinogenesis, since it has been shown that even at low doses these derivatives accumulate in DNA[1].

1. Green, T. and Hathway, D.E. [1978] Chem.-Biol. Interactions 22, 211-224.

581 IN VIVO VERSUS IN VITRO TREATMENTS FOR NITROSOMETHYLUREA MUTAGENESIS: A KEY TO THE MUTAGENIC LESIONS? Lynn S. Ripley, Laboratory of Molecular Genetics, National Institute of Environmental Health Science, Research Triangle Park, NC 27709.

Nitrosamides are carcinogens and mutagens in a variety of cellular and animal systems. The biological targets for these effects remain unidentified, confounded in part by the diversity of alkylated products found in DNA alone and by the possible importance of other cellular targets. Mutagenicity studies of nitrosamides in bacteriophage show these compounds to be particularly effective mutagens when phage are exposed within the host cell, but are non-mutagenic when free phage particles are treated. In contrast both methyl and ethyl methanesulphonate are mutagenic for free phage. An understanding of the requirement for in vivo treatments for nitrosamide mutagenesis might reveal the critical chemical lesions involved. Bacteriophage T4 posesses a sensitive, well-defined genetic system and is largely independent from its host with respect to DNA metabolism. These studies show that in vitro treatments of T4 particles with nitrosomethylurea (NMU) do not induce mutations but that in vivo treatments produce mutations at frequencies directly proportional to NMU concentration. G:C → A:T and A:T → G:C transitions are produced at high and moderate frequencies, respectively. Transversions and frameshifts are rarely induced. A phage-encoded error-prone repair system is not involved in the production of transition mutations induced by NMU. The mutagenic specificity of NMU is similar to that found for ethyl methanesulphonate. NMU is distinctly different from methyl methanesulphonate (MMS) in its mechanism of mutagenesis, since MMS depends upon the phage error-prone repair system.

Promotion, Cocarcinogenesis and Anticarcinogenesis

582 ORAL CONTRACEPTIVES AS PROMOTERS OF HEPATOCARCINOGENESIS. James D. Yager, Jr. and James E. Trosko, Dartmouth Medical School, Hanover, NH 03755 and Michigan State University, East Lansing, MI 48823. Liver cell adenomas and hepatocellular carcinomas have been reported in women taking oral contraceptives. Using an initiation-promotion protocol, we demonstrated that two oral contraceptive steroids (OCS), mestranol (M) and norethynodrel (N) can act as promoters of hepatocarcinogenesis (Cancer Res., 40: 3680-3685, 1980). The objective of the present study was to determine whether these two compounds can act as initiators. Detection of hepatocyte DNA damage by alkaline elution was used as an indication of initial potential. Intubation of female rats with 100 or 500 mg/kg of M, N, or ethinyl estradiol did not cause detectable DNA damage after 4 hours. Furthermore, none of these compounds caused a DNA repair response in rat hepatocytes in primary culture. The ability of M to initiate hepatocarcinogenesis was tested as follows: Forty female Sprague-Dawley rats were partially hepatectomized. Twenty-four hours later, ten rats each were intubated with corn oil (vehicle) or M at 100 or 500 mg/kg body weight. Ten additional rats received diethylnitrosamine (DEN) at 10 mg/kg, ip. Twenty-four hours after treatment, animals were shifted to phenobarbital diet to promote initiated cells. The animals were killed after 4 months and liver sections were analyzed histochemically for gamma glutamyl transpeptidase (γGT). Mestranol treatment did not cause a significant increase in the number of γGT foci while DEN treatment did. Other cellular effects of these agents were also tested. As detected with other known promoters, the OCS decreased metabolic cooperation in a V79 Chinese hamster cell culture system. These results strongly suggest that OCS lack the ability to initiate hepatocarcinogenesis and are in fact promoters. Supported by NIH Grant, CA 23916.

583 TPA INITIATION OF DNA SYNTHESIS OF CALCIUM-DEPRIVED T51B RAT LIVER CELLS IS MEDIATED BY CALMODULIN, CYCLIC AMP AND CYCLIC AMP-DEPENDENT PROTEIN KINASES, Alton L. Boynton, Alan Jones and James F. Whitfield, National Research Council, Ottawa, Canada K1A 0R6

The extracellular calcium ion is specifically required late in the prereplicative phase of regenerating rat liver, isoproterenol stimulated rat parotid gland in vivo or of cells of several established cell lines in vitro after serum stimulation. Furthermore, simply reducing the calcium concentration in the medium to 0.02 mM from the usual 1.82 mM reversibly reduced the fraction of cells labeled with ^{3}HTdR for 1 hr from 50-60% to only 15-25%. DNA synthetic activity resumed within an hour after raising the calcium concentration to 1.25 mM. This burst of DNA synthesis is mediated by calmodulin, cyclic AMP and cyclic AMP-dependent protein kinases. A wide variety of tumor promoters such as TPA, saccharin, cyclamates, phenobarbital, SDS, lithocholic acid, deoxycholic acid, mellitin, prostaglandins, EGF etc. all trigger DNA synthesis by calcium-deprived T51B rat liver cells. Moreover, this response to tumor promoters was mediated through calmodulin, cyclic AMP and cyclic AMP-dependent protein kinases because: 1) lanthanum, an inhibitor of calcium influx which also competes with calcium for cell surface binding sites prevents the response to TPA; 2) trifluoperazine, an inhibitor of $[Ca^{2+}]$-calmodulin function, blocks TPA- and saccharin-induced DNA synthesis, an effect which is reversed by addition of 10^{-6} mole/liter of purified rat calmodulin to the medium; 3) calmodulin itself stimulates the initiation of DNA synthesis; 4) calmodulin stimulates a cyclic AMP surge; 5) cyclic AMP itself stimulates the initiation of DNA synthesis; 6) a specific protein inhibitor of the catalytic subunits of cyclic AMP-dependent protein kinase prevents TPA- and saccharin from triggering DNA synthesis.

584 DO CHROMOSOMAL REARRANGEMENTS REPRESENT A RATE-LIMITING STEP IN CARCINOGENESIS? Anne R Kinsella, Paterson Laboratories, Christie Hospital & Holt Radium Inst., Manchester UK.
Radiation and chemical carcinogenesis might require at least two specific chromosomal events to coincide within a single target cell (1). Recent genetic and cytogenetic analysis of the effects of MNNG on V79 cells shows antipain to inhibit exclusively carcinogen-induced chromosome aberrations (2). No effect was observed on carcinogen-induced SCEs or forward mutagenesis to 6-thioguanine resistance. Conversely, TPA was shown to enhance MNNG-induced chromosome aberrations. This increase does not reflect an increase in aberrant cells, but a potentiation of chromosome rearrangement within the affected cells. Chromosomal aberrations might therefore represent a rate-limiting promotional step in carcinogenesis. With this in mind skin fibroblasts from Bloom's syndrome and q-D-deletion retinoblastoma patients have been adopted as models of promotion constituitive and initiated (deletion mutagenesis) cells, respectively. Theoretically these cells should require one or other of the processes of initiation or promotion, but not both. Preliminary data indicate that q-D-deletion retinoblastoma cells can be induced to grow on agar following treatment with TPA alone. In addition the effects of the carcinogen DMBA ± TPA ± inhibitors are being studied in mouse and human primary skin epithelial cultures with respect to chromosomal and morphological changes.
(1) Radman, M. and Kinsella, A.R. (1980) IARC Publn. 27, 75 - 90.
(2) Kinsella, A.R. and Radman, M. (1980) Proc.Natl.Acad.Sci.U.S.A., 77, 3544 - 3547.

585 SERUM FACTORS AFFECTING EXPRESSION OF THE TRANSFORMED PHENOTYPE. Merwin Moskowitz, David K. Moscatello and David K.S. Cheng, Purdue University, West Lafayette, IN 47907
3T3 cells do not grow in methocel suspension culture supplemented with calf or swine serum; however, swine serum and not calf serum stimulates a wave of DNA synthesis, maximal at 24 hrs, under these conditions. Calf serum inhibits the activity of swine serum. Heating at 65° inactivates the inhibitory activity of calf serum and increases the stimulatory activity of swine serum. Fractions obtained at 30-50% saturated ammonium sulfate contain the stimulatory activity of swine serum and the inhibitory activity of calf serum. Heating these fractions at 65° doubles the activity of the swine serum fraction, and causes the calf serum fraction to be stimulatory. 3T3 cells are contact inhibited in monolayer; swine serum and the stimulatory fractions increase the saturation density above that obtained with calf serum. The growth pattern in monolayer cultures with calf serum is typical of untransformed cells; with swine serum it is typical of transformed cells. Transformed cells grow in suspension culture more rapidly, and produce much larger colonies, with swine serum than with calf serum. Growth with swine serum is reduced after adding the inhibitory fraction of calf serum. The effects of the stimulating fractions are the same as those of tumor promoters (Adv. Canc. Res. 32: 1, 1980) under the conditions described. Our results suggest that there are components in sera having promoting and anti-promoting effects, and the activity of a given serum depends on the level of the respective components in it. If these components are active in vivo they may play a role in the development of cancer.

586 PHORBOL ESTER-INDUCED ANCHORAGE INDEPENDENCE AND ITS ANTAGONISM BY RETINOIC ACID CORRELATES WITH ALTERED SYNTHESIS OF PROCOLLAGEN, L. David Dion, Luigi M. DeLuca, Nancy H. Colburn, National Cancer Institute, FCRC, Frederick, MD 21701 and Bethesda, MD 20205
The JB-6 mouse epidermal cell line is being used as a model for promoter dependent preneoplastic progression. We have previously shown that tumor promoting but not nonpromoting phorbol diesters selectively inhibit the incorporation of ^{3}H-mannose or ^{3}H-proline into 180,000 MW glycoprotein. In contrast, retinoic acid enhances the mannose incorporation into the gp180 and antagonizes the TPA effect on simultaneous treatment at antipromoting concentrations. We have now identified the affected gp180 as procollagen by its sensitivity to purified collagenase digestion and formation of 95K fragments after pepsin treatment. After cloning the JB-6 cell population, the resulting clonal cell lines showed a spectrum of responsiveness to the promoting action of TPA that varied by 100-fold. Of eight clonal cell lines tested for the gp180 response, all showed two- to five-fold decreases in procollagen synthesis when treated with 1 to 100 ng TPA/ml, while 0.1 ng/ml (a concentration marginally active in promotion of anchorage independence) was only slightly effective. A major difference between the nonpromotable and promotable cell lines appears to be in the initial level of procollagen. SDS-polyacrylamide gels of ^{3}H-mannose labeled glycoproteins reveal that the 4 promotion responsive cell lines have 2 to 5% of their glycoproteins in the 180 K band while the 4 nonresponsive cell lines have 2 to 4 times that level of procollagen synthesis prior to TPA exposure. These studies suggest that initial levels of procollagen synthesis may influence a cells responsiveness to TPA perhaps by a differential effect on the amount of matrix material which is available for secretion.

587 PHORBOL ESTER INDUCED SPECIFIC CHANGES IN GANGLIOSIDE SYNTHESIS CORRELATE WITH PROMOTION OF ANCHORAGE INDEPENDENCE IN JB6 EPIDERMAL CELLS, Leela Srinivas and Nancy H. Colburn, Laboratory of Viral Carcinogenesis, NCI-FCRC, Frederick, MD 21701

In order to further investigate the nature and significance of plasma membrane effects of tumor promoting phorbol esters and in view of the differentiation associated changes in cellular gangliosides reported for human melanoma cells (Huberman et al., Cancer Res. 39:2618), we have investigated the role of ganglioside synthesis changes in promotion of tumor cell phenotype in JB6 epidermal cell lines. The tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate (TPA) produced after 24 hrs a 10-fold decrease in terminal 4-hr incorporation of 1-^{14}C-glucosamine into trisialogangliosides (G_T) and a two- to three-fold increase in precursor incoporation into the disialoganglioside G_{D1b}. The identity of G_T, the major ganglioside in JB6 cells, was established by demonstrating that the material incorporates palmitic acid (ruling out glycoprotein contamination), that it is resorcinol positive, that neuraminidase catalyzes its hydrolysis to G_{M1}, and that the ratio of sialic acid to hexose content is 1:1. The G_T synthesis decrease was observable from 4 to 48 hrs post treatment and occurred in response to tumor promoting but not non-promoting phorbol diesters. TPA was active for producing the G_T synthesis decrease at concentrations which are effective for promotion of anchorage independence in JB6 cells, namely at 0.1 to 10 ng/ml. Current studies are focused on determining the sensitivity of the G_T response to antagonism by the anti-promoter retinoic acid and on utilizing promotion or mitogen resistant variant cell lines to ascertain whether the G_T response might be a required event in either TPA-induced promotion or mitogenesis.

588 RELATIONSHIP OF POLYAMINE LEVEL ALTERATIONS TO TUMOR PROMOTION AND CELLULAR DIFFERENTIATION. * C.E.Weeks, A.Herrmann, F.Nelson and T.J.Slaga. ORNL, Oak Ridge,TN 37830

Evidence from our laboratory supports the theory that polyamine biosynthesis is a crucial factor in tumor promotion yet may be dissociable from promoter-induced proliferative aspects. For example in mouse epidermis 1) antiinflammatory steroids dramatically inhibit the tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate (TPA)-stimulated DNA synthesis and tumor incidence. They do not, however, greatly affect the changes in ornithine decarboxylase (ODC) activity or polyamine levels. On the other hand, 2) specific irreversible inhibitors of ODC markedly decrease the TPA-induced polyamine values, but have only slight effects on tumor formation. TPA treatment, however, does yield a dose-dependent increase in epidermal putrescine levels and putrescine levels in papillomas generated under above conditions are extremely high compared to normal epidermal values. Further, coincident application (ip) of exogenous putrescine enhances the TPA tumor level. Tumor promoting agents also dramatically affect cellular differentiation in mouse skin and several cell types in culture. We showed that TPA induces embryonic-like dark basal keratinocytes in mouse skin. Furthermore, we find that TPA induces epidermal transglutaminase (Tgase) activity. Maximal levels were 2-3 fold above control at 12-15 hr. Since Tgase(s) is involved in epithelial differentiation processes and the polyamines are substrates we suggest that polyamine involvement in differentiation may be a key aspect of tumor promotion. * Research sponsored by the Office of Health and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with the Union Carbide Corp.

589 THE EFFECT OF 12-O-TETRADECANOYL-PHORBOL-13-ACETATE (TPA) ON SPONTANEOUS AND UV- OR MNNG-INDUCED SISTER CHROMATID EXCHANGES, Alain Gentil and Georges Renault, Institut de Recherches Scientifiques sur le Cancer, B.P. n° 8, 94800 Villejuif, France.

It has been reported that, among its pleiotropic effects, the tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate (TPA) induces sister chromatid exchanges (SCE) which could be a reflection of increased recombinational activity and lead to the expression of a recessive mutational event (1). Contradictory results having been reported (2), we investigated the induction of SCE in either normal or in UV (2-5 Jm^{-2})- or MNNG (0.05 µg/ml)-treated V79 Chinese hamster cells. Our results showed that TPA induces SCE in normal cells even at low doses (0.1 to 1 µg/ml). An increase of approximately 50 % above the level of spontaneous SCE was observed. In UV-irradiated cells, TPA increased UV-induced SCE (15-20 %) when it was present after the UV-irradiation. This was not the case for MNNG-induced SCE. The protease inhibitor antipain inhibited TPA-induced SCE, but only partially inhibited the increased UV-induced SCE showing that TPA may act in two ways both leading to SCE induction. Although these data support the hypothesis that TPA may promote the segregation of a mutational event, the biological relevance of this theory is however questionable, since the TPA effects are small and it also has to be proved that SCE frequency is a real indicator of a recombinational activity.

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590 SELENIUM AND CANCER CHEMOPREVENTION, Milton V. Marshall and A. Clark Griffin, Biochemistry Division, The University of Texas System Cancer Center, Houston, TX 77030
Epidemiological studies of cancer incidence and levels of selenium indicate that higher levels of selenium may afford some protection against neoplasia. This hypothesis is further substantiated in animal systems where selenium supplementation lowers the tumor incidence following concurrent exposure to chemical carcinogens. Utilizing the carcinogen 2-acetylaminofluorene (AAF), we have investigated a mechanism of action for selenium. Male Sprague Dawley rats were fed a diet of 0.05% AAF for 14 weeks and for one group, the water was supplemented with 4 ppm selenium (as Na_2SeO_3). In 20 weeks, the hepatic tumor incidence of rats fed the selenium-supplemented diet was 4/14 compared to 9/13 for rats receiving AAF alone. In vitro studies on the metabolism of AAF were also investigated using a liver microsomal fraction to study the effects of selenium on AAF metabolism. Selenium administration inhibited N-hydroxylation of AAF while ring hydroxylation was enhanced. A decreased binding of AAF and N-OH AAF to calf thymus DNA was also observed. Dietary selenium administration also enhanced the glucuronide conjugation of AAF metabolites. Selenium thus appears to decrease AAF carcinogenicity by enhancing detoxification pathways (ring hydroxylation and glucuronide conjugation) and decreasing activation (N-hydroxylation). The net result is to decrease the formation of N-OH AAF and to inhibit the further metabolism of N-OH AAF to its ultimate carcinogenic metabolite as determined by DNA binding.

591 SPECIFIC BINDING OF PHORBOL ESTER TUMOR PROMOTERS TO INTACT PRIMARY EPIDERMAL CELLS FROM SENCAR MICE. V.Solanki & T.J.Slaga. Biol.Div.,Oak Ridge Nat'l Lab., Oak Ridge, Tennessee 37830.
Binding of [20-^{3}H]phorbol 12,13-dibutyrate ([^{3}H]PDBu) to intact living epidermal cells in monolayer culture was characterized. At 37°C the maximum specific [^{3}H]PDBu binding (displaced by 30 uM unlabeled PDBu) was attained within 15-20 min followed by a rapid decline (down regulation) of radioactivity. Prior exposure of cells with TPA but not with phorbol for 2 h at 37°C, caused about 55% reduction in the number of measurable binding sites for [^{3}H]PDBu. In addition, the down regulation was found to be temperature sensitive as no loss of radioactivity occurred by 1 h at 4°C. The specific binding of [^{3}H]PDBu at 4°C reached equilibrium within 15-20 min, was saturable and freely reversible. At equilibrium, epidermal cells were found to contain ~1.2 x 10^5 binding sites per cell which had a dissociation constant (K_D) for [^{3}H]PDBu of 10 nM. Specificity of binding was demonstrated by the observation that the biologically active phorbol esters 12-0-tetradecanoyl-phorbol-13-acetate (TPA) and 12-deoxyphorbol-13-decanoate (DPD) inhibited the binding, whereas the inactive parent compound phorbol and non-phorbol promoter anthralin did not have any effect. The abilities of these compounds to inhibit binding was in direct correlation to their tumor promoting activities. Epidermal cells exposed to retinoic acid (RA) or fluocinolone acetonide (FA) for 24 h had similar [^{3}H]PDBu binding characteristics as untreated cells indicating that RA- or FA-induced inhibition of tumor promotion is not mediated through alterations in the phorbol ester binding sites. * Research sponsored by the Office of Health & Environ. Res., U.S.Dept. of Energy, under contract W-7405-eng-26 with Union Carbide Corporation.

592 SPECIFIC BINDING OF 3H-TPA TO NORMAL AND TRANSFORMED HUMAN HEMATOPOEITIC CELLS,Thomas D.Gindhart and Bakul Dalal,National Institutes of Health, Bethesda, MD 20205
A phorbol diester receptor assay for specific binding of 3H-TPA to intact cells was developed in order to study the influence of pharmacodynamic parameters on receptor dependent biologic responses.Specific binding of 3H-TPA to normal and malignant human hematopoeitic cells ranged up to 80% of total in a binding assay utilizing medium containing 5 mg/ml BSA carrier protein and rapid centrifugation in unsiliconized borosilicate glass tubes. Human PBMC, PBL, PMN and two leukemic cell lines bound specifically 1-4 x 10^5 molecules of 3H-TPA per cell with K_d's from 1-6 nM. Platelets bound 3H-TPA with high affinity while RBC's showed no specific binding. K562, a CML cell line isolated in blast crisis, showed variation in binding related to phase of growth. A second higher affinity binding site with a K_d of 70-100 pM was also detected in cells at saturation density. HL-60, a promyelocytic cell line, and it's adhesion-resistant variant, TG 20, both possessed 2 x 10^5 binding sites with similar K_d's. Scatchard analysis of binding data utilizing the direct linear plot, best fit by trial and error and Michaelis-Menten kinetics yielded similar values in each case. Hill plots and cold ligand displacement studies indicated no positive or negative cooperativity. Parallel assays with 3H-PDBu and 3H-TPA of nylon wool purified lymphocytes showed 26 x 10^3 binding sites/cell for both ligands and K_d's of 9.7 nM for PDBu and 0.3 nM for TPA. These results suggest that 1: Transformed cells do not differ markedly from normal cells in number or affinity of TPA binding sites 2: Loss of responsiveness to TPA need not depend on a change in binding site number or affinity 3:More than one specific binding site for TPA can occur in cells.

593 ROLE OF ORNITHINE DECARBOXYLASE IN HEPATOCARCINOGENESIS, Jack W. Olson, College of Pharmacy, University of Kentucky, Lexington, Kentucky 40506.

Data from studies of the mouse epidermal carcinogenesis model suggest that the induction of ornithine decarboxylase (ODC), the initial and rate limiting step in the biosynthesis of polyamines, is a specific and essential event in the phorbol ester promotion of skin tumors. Olson and Russell have recently reported that the Solt and Farber multi-stage chemical hepatocarcinogenesis regimen resulted in the prolonged activation of ODC and increased spermidine levels which appeared to be coupled to the development of pre-neoplastic nodules. To further examine the significance of this prolonged activation of ODC, the potent, site specific, enzyme-activated, irreversible ODC inhibitor, α-difluoromethylornithine (DFMO), was administered to rats subjected to the Solt and Farber hepatocarcinogenesis regimen. Rats were given diethylnitrosamine (DEN) in one dose (200 mg/kg, i.p.) followed by 2 weeks of dietary 0.02% 2-acetylaminofluorene starting at day 14 after DEN, followed by partial hepatectomy (PH) on day 21. Treatment consisted of either drinking water or DFMO in the drinking water starting at day 19 and continued till sacrifice. 1% DFMO treatment (mean daily intake of 800 mg/kg body wt) did not alter ODC or S-adenoxyl-L-methionine (SAM-DC) activity at 1, 7 or 25 days after PH. 3% DFMO treatment (3000 mg/kg/day) decreased ODC activity by 76% and increased SAM-DC activity by 192% at 11 days after PH. DFMO treatment did not alter the marked elevation of γ-glutamyltranspeptidase activity, a putative preneoplastic marker, nor did it appear to significantly alter preneoplastic nodule formation. Additional morphological evaluation and DFMO treatment regimens are in progress to further examine the role of ODC in the development and progression of preneoplastic liver nodules.

594 MODULATION OF 3-HYDROXYXANTHINE-INDUCED SISTER CHROMATID EXCHANGE IN CHINESE HAMSTER CELLS BY CIS-RETINOIC ACID. Suresh C. Jhanwar, Gerhard Stöhrer, and R.S.K. Chaganti. Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, N.Y. 10021. Retinoic acid, (RA) an antioxidant, acts as an inhibitor in experimental carcinogenesis. 3-hydroxyxanthine (3-HX) is a carcinogen which produces sarcomas and other tumors in rats. We have studied sister chromatid exchange (SCE) induction by 3-HX and its modulation by RA in chinese hamster cells in vivo and in vitro. Five male hamsters weighing between 40 and 45gms were used for the in vivo experiments. Animals 1 and 2 received BrdU only and served as controls. Animals 3 and 4 received BrdU and 2mg of 3-HX; animal 4 was also fed RA during four days prior to administration of carcinogen. Animal 5 received BrdU and 6mg of 3-HX. Bone marrow (BM) cells of the control animals (1,2) had mean SCE of 3.58 and 4.43 per cell respectively, while BM cells of experimental animals (3,4,5) exhibited mean SCE of 4.97, 4.27, and 7.46 per cell respectively. Fibroblast cells in culture derived from the lung of an adult male hamster were used in the in vitro experiments. Five T75 flasks with cultures in log phase were treated in the following way. Flasks 1 and 2 received BrdU only and served as controls. Flasks 3 and 4 received BrdU and 420 and 500μg respectively of 3-HX. Flask 5 received BrdU, 500μg of 3-HX, and 0.075mg of RA. Cells from control flasks (1,2) had mean SCE of 12.26 and 10.08 per cell respectively. Cells from experimental flasks (3,4,5) exhibited mean SCE of 21.23, 32.3 and 24.5 per cell respectively. These results demonstrate that (a) 3-HX induces a significant and tissue specific increase in SCE and (b) RA has no synergistic effect on SCE induction by 3-HX in vivo as well as in vitro, but instead has an inhibitory effect on SCE induction by 3-HX in at least one cell type, namely, the cultured fibroblast.

595 MODULATION OF THE EFFECTS OF RETINOIDS ON THE GROWTH AND MORPHOLOGY OF C3H/10T½ CELLS BY SERUM AND AN INHIBITOR OF cAMP-SPECIFIC PHOSPHODIESTERASE. Lawrence J. Mordan, and John S. Bertram, Cancer Drug Center, Roswell Park Memorial Institute, Buffalo, N.Y. 14263

Retinoids can reversibly inhibit neoplastic transformation induced by carcinogens in 10T½ cells and also increase the adhesion of these cells to the substrate. Neoplastic transformation typically involves the loss of contact inhibition, reduced serum requirements, reduced adhesion, cytoskeletal disorganization, decreased cell spreading, and decreased cAMP levels. In order to examine inhibition of carcinogenesis, we have measured retinoid-induced changes in these parameters in 10T½ cells. Retinyl acetate and retinoic acid a) decrease the saturation density in a dose-dependent manner, b) increase the spreading of cells, c) decrease the underlapping d) increase the adhesion of cells to the substrate, and e) double the frequency of microfilament bundles (MFB). Reducing the serum concentration from 10% to 2.5%, induced these same changes, except for e, and the effectiveness of retinoids to induce these changes is decreased. Because reduced serum levels may cause an increase in the intracellular cAMP concentration, these results suggest that retinoids may effect cyclic nucleotide metabolism. Treatment with the cAMP-specific phosphodiesterase, inhibitor Ro 20-1724/1 induces a 50% increase in b, but did not effect e. Simultaneous treatment with retinyl acetate and Ro increased b more than either drug alone, while e was unchanged from the density induced by retinoid treatment alone. Ro inhibits growth of transformed 10T½ cells only when cocultivated with normal 10T½ cells. These results suggest that retinoids induce more effective interactions, enhance the accumulation of cAMP, and maintain the normal phenotype. Supported by USPHS Grant CA25484.

596 INTERMEDIATE TRANSFORMING PHENOTYPES INDUCED BY POTENTIAL TUMOR PROMOTERS IN NORMAL AND tsRSV-INFECTED CHICKEN EMBRYO CELLS. Masatoshi K. Owada, Hiroshi Yamasaki*, Peter Donner, and Karin Moelling, Max-Planck-Institut für Molekulare Genetik, Ihnestrasse 63, 1000 Berlin 33. *International Agency for Research on Cancer, Lyon, France.

12-O-tetradecanoylphorbol-13-acetate (TPA) induced morphological alterations in tsRSV-infected chicken embryo cells (CEC) at the nonpermissive temperature (41°C), as well as in normal CEC. Biological and biochemical effects of TPA on tsRSV-CEC have been investigated: 1) Stimulation of cell-proliferation accompanied by criss-cross growth. 2) Inhibition of colony formation in soft-agar of tsRSV-CEC at 36°C at the concentration of 1-100 ng/ml TPA. 3) Enhancement of hexose-uptake in tsRSV-CEC at 41°C, which reaches 50% of the level seen at 36°C. 4) TPA is known to stimulate transiently mouse epidermal ornithine decarboxylase (ODC) activity. It stimulated ODC-activity in normal (20fold) and in tsRSV-CEC (2fold). The activity was found to be 50-100fold higher in transformed CEC than in normal CEC. Rapid recovery of the activity in tsRSV-CEC was observed to precede recovery of morphological transformation after down-shift (36°C). 5) The protein kinase activity associated with $pp60^{src}$, the protein encoded by the RSV-sarcoma gene, and its degree of phosphorylation did not change with TPA. The protein kinase activity of the normal cell homologue, $pp60^{sarc}$, remained constant. 6) Protein kinase activity of partially purified $pp60^{src}$ was not influenced by TPA in an in vitro assay. 7) Total amount of phosphorylated tyrosine-amino acids remained unchanged in TPA-treated cells. It appears that TPA induces a transformation unrelated to $pp60^{src}$ or its normal cellular homologue. A test of TPA on tumorigenicity of tsRSV in chickens is in progress.

597 TUMOR PROMOTER INDUCED RETRACTION OF NEURONAL PROCESSES IN MURINE NEUROBLASTOMA. William H. Gibson, Jr. and Shari L. Burack, Douglass College - Rutgers University, New Brunswick, N.J. 08903

The affect of several phorbol diester tumor promoters on differentiated neuroblastoma cells (NB15) was determined. Treatment of NB15 cells with serum free medium containing five micrograms of 5-bromodeoxy uridine per milliliter of culture medium, resulted in the morphological differentiation of greater than eighty percent of the cells in the population. A cell is considered differentiated, if it possesses one neuronal process fifty microns in length. Biochemical differentiation results in an increase in the specific activity of one or more neuron specific enzyme. Greater than eighty percent of the cells in the cultures remain viable after seventy two hours of induction in this medium. Treatment of differentiated cells with phorbol diester compounds, which are active promoters of tumors in mouse skin, result in retraction of neuronal processes. The most effective phorbol diester was 12-tetradecanoyl phorbol 13 acetate (TPA). The optimum concentration for the dedifferentiation of NB15 was 150 nanogram of TPA per milliliter of culture medium. The percent morpholobical differentiation decreased significantly within three hours after treatment, and was less than the spontaneous level after twenty-four hours of treatment. The specific activity of acetylcholinesterase decreased significantly during this period.

In Vitro and in Vivo Carcinogenesis

598 THYROID HORMONE MODULATES NEOPLASTIC TRANSFORMATION *in Vitro*, Carmia Borek, & Duane L. Guernsey, Columbia University, College of Physicians & Surgeons, New York, N.Y. 10032

Neoplastic transformation *in Vitro* by X-rays (1) has established the direct oncogenic potential of radiation in mammalian cells. While several agents (review see 2) modulate radiation induced transformation little is known whether hormones, normally present in mammalian serum can affect this response.

Using short term cultures of diploid hamster embryo cells, and the heteroploid mouse cell line C_3H 10T½ we have investigated the effect of thyroid hormone triiodothyronine (T3) on transformation. We have found that in both cell types the removal of thyroid hormone from serum supplemented culture medium (using AG1-X10 resin) inhibits X-ray induced transformation while having no effect on cellular growth rate or survival. When T3 alone is added to thyroid depleted medium at concentrations of 10^{-7}M transformation frequency is expressed at a similar frequency as in the presence of culture medium supplemented with untreated serum. Ongoing work indicates that exposing the cells to T3 ranging from 10^{-11}M to 10^{-7}M results in transformation which is dose dependent. Thyroid hormone is therefore necessary for the induction and/or expression of X-ray induced transformations. We are now investigating its role in chemically and virally induced transformation.

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599 RESPONSE OF PROGRESSOR AND REGRESSOR CHICKENS TO ROUS SARCOMA VIRUS.
John A. Thoma, Craig Whitfill, J. Allen, E. Weck, N. R. Gyles and L. Patterson,

University of Arkansas, Fayetteville, Arkansas. The Arkansas chicken model for Rous sarcoma regression has been genetically selected for that trait for over 10 generations. The regressor (R) line exhibits about 85% regression while the progressor (P) line develops about 85% fatal tumors. From earlier studies, it was hypothesized that the difference between the two lines was the early recognition and response of the R line to the RSV challenge. As one test of this hypothesis we measured the responsiveness of the P and R peripheral leucocytes to nonspecific mitogen and specific tumor antigen signals. The average leucocyte blastogenesis as measured by ^{3}H thymidine incorporation was two times greater for PHA stimulated leucocytes than for con A stimulated lymphocytes. R leucocytes were two times more responsive than P leucocytes at optimum doses of mitogens. P leucocytes did not respond in vitro to tumor antigens at any time during the entire course of tumor growth but the R leucocytes showed an enhanced stimulation about 2 weeks after virus challenge. The significance of the enhanced sensitivity of R leucocytes to these three stimuli is clouded by the large variation of response within sublines.

Sera from regressor chickens yielded two low molecular weight components that exhibit RSV neutralizing activity. The components have molecular weights of 750 and 150 daltons. The factors achieve maximum levels at 11 days post RSV challenge and disappeared when the tumors regressed, but reappeared several days after a secondary challenge. Significant levels of virus neutralizing factors were not found in progressor sera.

600 MAMMARY CELL MEDIATED MUTAGENESIS OF MAMMALIAN CELLS, Michael N. Gould, Department of Human Oncology, WCCC, University of Wisconsin, Madison, WI 53792

We are utilizing a system in which cultured rat mammary gland cells are employed to activate chemical procarcinogens to active mutagens. Mutagenesis is tested in co-cultured Chinese hamster V-79 cells. The loci tested are mutation to the resistance to ouabain and/or 6-thioguanine.

Cells from mammary tissue are divided into populations enriched for either parenchymal, or stromal cells by selective adhesion to plastic. We have found both inter- and intra-organ specificity in the activation of procarcinogens by these cells. DMBA, a very potent in vivo mammary carcinogen is activated by both stromal and parenchymal mammary cells. Benzo (a)-pyrene, a weak mammary carcinogen, can be activated by the stromal cells but not by the parenchymal cells. The potent hepatocarcinogen, aflatoxin B_1, cannot be activated by either mammary cell population.

601 POTENTIATION OF MURINE VIRAL LEUKEMOGENESIS BY CHEMICAL CARCINOGENS.
Radmila B. Raikow and James P. OKunewick,
Allegheny-Singer Research Corp. Pittsburgh, Pa. 15212

Our data indicate that a single i.p. injection of 2 mg. methyl methane sulfonate (MMS), 500 ug benzo[a]pyrene (BP) or 500ug 7,12 dimethyl benzathracene (DMBA) can potentiate the leukemogenic action of a subsequently injected low dose of Friend leukemia virus in mice. The optimal time interval between the injection of chemical carcinogen and virus varied according to the chemical used. Thus this interval appeared to be 5 hrs for MMS but 2 and 8 days for BP and DMBA, respectively. Two mouse strains, the virus-sensitive SJL/J and the relatively virus-resistant B10SJF1 hybrid were used and induced leukemia was diagnosed by splenomegaly, lymph node and thymus gland enlargement and elevated white cells in the peripheral blood. The virus potentiating effect was seen more clearly in the hybrid because the virus and the chemical carcinogen injected alone had relatively small effects on the survival of these mice. With DMBA there was also some evidence of a potentiation of the endogenous lymphocytic leukemia virus in SJL/J mice. Attempts to elucidate the mechanism of the virus potentiation indicate that immunosuppression is not involved but that at least with some of the chemical carcinogens, repairable damage to the virus target cell is involved. Supported by the Department of Energy.

602 RELATIONSHIP BETWEEN CHEMICALLY-INDUCED AND SPONTANEOUS LYMPHOMA IN RF MICE, Maureen M. Goodenow, Maria L. Duran-Reynals and Frank Lilly, Albert Einstein College of Medicine, Bronx, NY 10461

When 3-methylcholanthrene (MCA) is applied percutaneously to mice of various inbred strains, one of two neoplastic responses may be manifested which appear to be strain specific and influenced by the genotype at the *Ah* locus. After treatment with MCA, mice homozygous or heterozygous for the dominant Ah^b allele show elevated levels of aryl hydrocarbon hydroxylase (AHH) activity and a high incidence of skin tumors. AHH-noninducible mice, homozygous for the recessive Ah^d allele, develop thymic lymphoma in response to MCA treatment. Specifically, RF/J mice (Ah^d/Ah^d) respond to MCA with an exceptionally high incidence of lymphoma (more than 90% die from thymic lymphoma by six months of age while fewer than 10% of untreated RF mice develop the disease at a corresponding age). We have chosen the RF strain of inbred mice in order to study the relationship between MCA-induced and spontaneous lymphoma and to examine what, if any, is the role of endogenous virus in the etiology of these tumors. A number of tissue culture cell lines and transplantable tumors have been established from different RF lymphomas of both chemically-induced and spontaneous origin. These transformed cells have been examined for the presence of normal thymocyte differentiation antigens (*Thy-1*, *Lyt-1*, and *Lyt-2*) as well as some antigens (gp69/71 and p30) associated with expression of endogenous murine leukemia virus genomes. Results obtained from the use of monoclonal antibodies in conjunction with the fluorescence-activated cell sorter and from electron microscopy will be presented.

603 FACTORS INFLUENCING THE INDUCTION AND EXPRESSION OF ONCOGENIC TRANSFORMATION IN HUMAN DIPLOID CELLS, Robert J. Zimmerman and John B. Little, Laboratory of Radiobiology, Harvard School of Public Health, Boston, MA 02115

We have studied oncogenic transformation in human diploid fibroblasts by a modification of the technique described by Milo and DiPaolo (Nature 274: 130, 1978). Synchronized cells were treated in mid S-phase with N-acetoxy-2-acetylaminoflourene, and transformants were identified by scoring for anchorage-independent growth at various population doublings post-treatment. Transformation was observed following treatment with both toxic and non-toxic doses of the carcinogen, and was dose-dependent over the range of 1-35μM in the medium. We have examined several experimental parameters which influence the frequency of this event: 1) the means of synchronization of the cell population prior to S-phase treatment, 2) the choice of selective media post-treatment, 3) the number of population doublings allowed post-treatment prior to plating for suspension growth, and 4) the influence of cell number seeded per dish on the scored frequency.

DNA repair experiments using labelled carcinogen indicate that synchronous cells treated in mid-S phase remove DNA-adducts less efficiently than cells treated under density-inhibited growth conditions. The presence of these persistent adducts may partially explain the high transformation frequencies observed in S-phase treated cells.

604 **MECHANISMS OF MAMMARY CARCINOGENESIS IN VIVO** D. N. Mhaskar, J. M. Raber, M. J. W. Chang, and S. D'Ambrosio. Depts. of Pharmacology and Radiology, The Ohio State University, Columbus, Ohio 43210

In order to elucidate the underlying mechanisms for chemical carcinogenesis in rat mammary gland, we quantitated: (a) the number of alkali labile sites (alkyl phosphotriesters) by alkaline sucrose gradient sedimentation coupled with a fluorescent method for detecting non-radiolabeled mammary DNA; (b) the number of N-3 and N-7 alkylpurine sites by a selective depurination assay followed by sedimentation of formamide denatured nonradiolabeled DNA through neutral sucrose gradients; and (c) all adducts using radiolabeled carcinogens and chromatography. We determined the initial level of DNA damage and followed the fate of such damage over a period of 7 days following injection with N-ethyl-N-nitrosourea (ENU) (carcinogenic) and non-carcinogenic N-benzyl-N-nitrosourea (BNU) in female Sprauge-Dawley rats. Our data showed that: (a) the initial level of damage induced was higher in the mammary gland of the 50 day than in the 30 day old rat; (b) alkaline labile damage induced by ENU persisted in the 30 day old rat while it appeared to be lost in the 50 day old rat; (c) initial level of damage by BNU was less than that induced by ENU; (d) loss of damage induced by ENU appeared to be faster in the Long Evans than in the Sprauge-Dawley rat strain. These data relate the induction and persistance of DNA damage induced by alkylator in vivo to mammary gland carcinogenesis as a function of agent, age, gland development and strain of the rat. Supported by NCI contract # 84226.

605 EARLY EFFECTS OF CARCINOGENS ON COLONIC CYCLIC NUCLEOTIDE METABOLISM. F. R. DeRubertis and P. A. Craven Dept. Medicine, VA Hospital and Univ. of Pgh, Pgh, PA 15240

The present study examined the acute effects of a single i.r. dose of the colon carcinogens 1, 2 dimethylhadrazine (DMH) or nitrosoguanidine (MNNG) on [^{3}H] thymidine incorporation into DNA ([^{3}H] Tdr), cAMP and cGMP in rat distal colonic mucosa. DMH initially inhibited [^{3}H] Tdr (12 and 24 hours) at which time there were no changes in cAMP, cGMP or cAMP dependent protein kinase (PK) activity compared to vehicle control. At 3 and 5 days after DMH, [^{3}H] Tdr was increased. Tissue cAMP content and the activity ratio of cytosolic PK were reduced, whereas cGMP did not change. The % type I isoenzyme of PK, a form associated with increased proliferative activity in other tissues, was increased. DMH induced increases in [^{3}H] Tdr and the % type I PK were observed in cells isolated from both the superficial and lower crypt. DMH induced changes in [^{3}H] Tdr, DNA, cAMP and PK were transient, and returned to control by 10 days. Low dose i.r. MNNG increased cGMP, without altering cAMP or [^{3}H] Tdr. In short term cultures of rat colon, dibutyryl cAMP, PGE_2 and vasoactive intestinal peptide increased cAMP and suppressed [3H] Tdr in both tissue with and without exposure to DMH *in vivo*. By contrast, dibutyryl cGMP and 10 μM MNNG, which increased cGMP but not cAMP 2-fold, did not affect [^{3}H] Tdr acutely *in vitro*. The present data are consistent with an inhibitory influence of cAMP on the proliferative activity of colonic epithelium and suggest that reduction of cAMP and cAMP dependent PK activity could play a permissive role in the enhancement of [^{3}H] Tdr observed 3 to 5 days after DMH. The actions of cGMP remain to be defined.

606 COMPARISON OF LECTIN AGGLUTINABILITY OF MAMMARY TUMOUR CELLS WITH METASTATIC COLONISATION POTENTIAL, Janet E. Price and David Tarin, Department of Histopathology, John Radcliffe Hospital, University of Oxford, Oxford OX3 9DU

In previous work in this laboratory it has been found that about 50% of primary (i.e. naturally-occurring) murine mammary tumours are capable of heavily colonising the lungs after intravenous inoculation, although spontaneous metastasis is infrequent. In this investigation lectin agglutinability has been used as a means of comparing the surface composition of cells from tumours which can heavily colonise the lung with that of cells from tumours which cannot. Dispersed monocellular suspensions obtained by collagenase digestion of the tumours were re-inoculated via the tail vein into the donor and batches of 5 syngeneic animals, and aliquots from the same suspension were studied for lectin agglutinability on the same day. Debris and red cells were removed from the aliquots by density-gradient centrifugation on Percoll and the rate and degree of agglutinability assessed by measurement of the disappearance of single cells from suspension with a Coulter counter linked with a C-1000 channelizer. It was found that agglutinability with the lectins Concanavlin-A and Wheatgerm agglutin bore no relationship to the pulmonary colonisation potential of the primary mammary tumour. However, cells from disaggregated secondary deposits of tumours which manifested high colonisation potential were consistently less agglutinable than the cells of the primary tumours from which they were derived. Investigation now in progress seeks to establish whether this difference is due to a selection process during dissemination or to site-induced changes in the cells which lodge in the lung.

607 A METHOD FOR CONTINUOUSLY MONITORING THE EFFECT OF TUMOR PROMOTERS ON ORNITHINE DECARBOXYLASE ACTIVITY IN GROWING HUMAN TISSUES. Lyle Arnold, Environmental Carcinogenesis Laboratory, Dept. of Community Medicine, UCSD, School of Medicine, La Jolla, Ca. 92093.

In the last several years a striking relationship has been found between the induction of ornithine decarboxylase (ODC) activity and conditions which promote tumor formation. One particularly sensitive system is mouse skin epidermis treated with the potent tumor promoter TPA (12-O-decanoylphorbol-13-acetate). We have designed a system which permits us to monitor ODC activity continuously in viable human tissues. Typically 50 mgs of human foreskin grown on sterile pig skin dermis is incubated with 3.6 ml Eagle's MEM plus 10% calf serum containing $^{14}CO_2$ ornithine (0.045 nM, s.a. 13.5 mCi/mmol) and carbonic anhydrase (300 μg/ml). As radioactive ornithine is metabolized by the tissue, $^{14}CO_2$ is released. In the presence of carbonic anhydrase the released $^{14}CO_2$ exchanges rapidly with a stream of 5% CO_2 in air which is played on the surface of the media. Effluent air is then bubbled through ethanolamine dissolved in scintillation fluid to collect the expired $^{14}CO_2$. Using this procedure we have found that the 1 μg/ml TPA in fresh media increases the activity of ODC in human skin from 25 pmol CO_2/30min to 180 pmol CO_2/30min at nine hours after exposure-an increase of more than seven fold. The activity which peaks sharply declines back to the control levels after 48 hrs. In contrast, fresh media alone causes an increase of only two fold in activity and peaks at 1 to 3 hrs after exposure, declining to plateau level within 5 hrs. These results indicate that this sensitive system may be useful for evaluating promoters directly on human tissues. Additional effects of TPA and other promoters will be presented. Supported by the UCSD Cancer Center Research Development Core Service NIH 5 P30 CA23100.

608 DNA METHYLATION AND S-ADENOSYLMETHIONINE (SAM) METABOLISM IN SPONTANEOUS AND CHEMICALLY INDUCED HEPATOCELLULAR CARCINOMA IN B_6C_3 MICE, Jean-Numa Lapeyre, Margaret S. Walker, M.C. Liau and Frederick F. Becker, Section of Experimental Pathology, M. D. Anderson Hospital and Tumor Institute, Houston, TX 77030

DNA methylation in eukaryotic cells has been implicated in the regulation of gene activity and differentiation. Previous studies in this laboratory have shown that the levels of endogenous DNA methylation were abnormally low in hepatocellular carcinoma (PHC) induced in the rat with N-acetylaminofluorene (AAF) or diethylnitrosamine (DEN). These studies have been extended to examine the levels of DNA methylation in B_6C_3 mice which display the parental strain susceptability to chemically induced carcinoma (C57Bl/6N) and partial expression of spontaneous PHC (C3H/HeN). Deficient DNA methylation was observed in spontaneous PHC in B_6C_3 and C_3H/HeN, and in the PHC induced by 3'methyl-4-dimethylaminoazobenzene, chlordane, AAF and DEN in B_6C_3 or C57Bl/6N. Since the undermethylation of tumor genomes might be related to a deficiency in PHC of the enzyme DNA methyltransferase or enzymes involved in SAM metabolism, the levels of these enzymes and isozymic forms were examined in PHC, normal, and background hepatic tissues. No abnormalities were observed in the activity or isozymes of SAM synthetase, S-adenosylhomocysteine (SAH) hydrolase, SAM decarboxylase, or SAM-homocysteine methyltransferase nor availability of SAM and its ratio to SAH, a competitive inhibitor of methylation reactions. In PHC, however, the level of DNA methyltransferase activity was found to be similar to that in quiescent background and normal hepatic tissues, while the rate of DNA synthesis in PHC was elevated compared to these quiescent tissues but less than in regenerating liver which had far greater levels of DNA methyltransferase activity. These results suggest that this key enzyme may be limiting in cells which escape into uncontrolled growth. (Supported by NIH Grant CA 20657).

609 THRESHOLD MUTAGENIC CONCENTRATIONS OF CHEMICAL CARCINOGENS, Tamara E. Awerbuch, Department of Biology, Massachusetts Institute of Technology, Cambridge, Mass. 02139

A mathematical model was developed describing a diffusion bioassay for detecting mutagenic concentrations of chemical carcinogens. As a result two equations were obtained: (a) An equation that enables us to calculate the decay time of the mutagen and; (b) A functional relationship between the radius at each point of the petri dish and the time average concentrations of the mutagen at this point. From knowledge of the radius (r_{mut}) of the mutagenic zone the lower concentration (C_{mut}) at which the mutagenic response is observed can be calculated. Via this method values of C_{mut} were obtained for N-methyl-N'-nitro-N-nitrosoguanidine (NG), N-methyl-N-nitrosourea, (MNU), ethylmethanesulfonate (EMS), acetoxy dimethylnitrosamine (AcDMN), nitrosomorpholine (MM) and nitrosopyrrolidene(NP). We compared our results to those obtained with the standard plate assay developed by Ames. The statistical analysis of the data showed that the standard plate assay is not adequate for determining thresholds, while it was possible to show the existence of thresholds via the diffusion bioassay. If we use the concept of the threshold to rank the mutagenic potency of chemical carcinogens we get the following ranking: NG>NP>MNU>NM>AcDNM>EMS.

610 AN EPIDERMAL IN VITRO MODEL FOR THE IN VIVO TPA INDUCED PROSTAGLANDIN SYNTHESIS, ODC ACTIVITY AND DNA SYNTHESIS.* S.M.Fischer & G. Furstenberger, Biol. Div., ORNL, Oak Ridge Nat'l. Lab., Oak Ridge, TN and Deutsches Krebsforschungszentrum, Heidelberg, Germany.

Topical treatment of adult mouse skin with TPA results in the early induction of prostaglandin (PG) synthesis, followed by the induction of ODC activity and finally hyperplasia. Since an in vitro model of these events would facilitate more a detailed investigation of these early events, i.e., TPA induced release of arachidonic acid and PG synthesis, primary cultures of neonatal epidermal cells from NMRI mice were established. Treatment with 1 ug/ml TPA resulted in a 60% increase in arachidonic acid release and a 250% increase in PG synthesis, followed by a 5-fold induction in ODC activity and subsequent 6-fold stimulation of DNA synthesis. Further, the PG inhibitor indomethacin (10^{-4}M) was demonstrated to inhibit not only PG synthesis but also ODC and DNA synthesis by 100, 40 and 60% respectively. This inhibition could be overcome by the addition of 10^{-6} either PGE_2 or $PGF_{2\alpha}$ in the same manner as has been reported in vivo for this mouse. These results demonstrate both the obligatory nature of the PG's to certain biochemical events evoked by TPA as well as the establishment of an in vitro model for some of the early events seen in vivo. * Research sponsored jointly by Deutsches Krebsforschungszentrum, Heidelberg, Germany and the U.S. Dept. of Energy under contract W-7405-eng-26 with Union Carbide Corporation.

611 A POSSIBLE APPROACH FOR CORRELATING HISTOPATHOLOGICAL ALTERATIONS TO MUTATION AND TRANSFORMATION IN VITRO, Makito Emura, Hans-Bernhard Richter-Reichhelm and Ulrich Mohr, Department of Experimental Pathology, Hannover Medical School, Hannover, FRG

When Syrian hamster fetuses were exposed to DEN in utero, they developed a great number of metaplastic and dysplastic changes, as well as papillary tumours in the tracheal epithelium in later life (Invest. Cell Pathol. 1980). Fetal tracheal explants taken after DEN-administration to the mothers and cultured for 4 weeks developed metaplastic and dysplastic alterations in the respiratory epthelium (Cancer Lett. 1978). Improved culture conditions and application of scanning electron microscopy enabled constant observation of in vitro morphology and facilitated the investigation of the epithelium in question (Exp. Path. 1979, Zbl. Bakt. Hyg. B. 1980). In vitro exposure of tracheal explants to B(a)P also showed alterations such as metaplasia and dysplasia which could be detected by SEM. Studies with directly-acting compounds such as MNNG on morphologically selected non-fibroblastic fetal hamster cell lines showed transformation and tumorigenicity (EACR, 1979). We are particularly interested in examining the extent to which morphological alterations such as metaplasia and dysplasia may be correlated to mutation and transformation in cell culture systems.

612

NEOPLASTIC TRANSFORMATION OF DIPLOID HUMAN FIBROBLASTS BY CARCINOGENS. J. J. McCormick, K. C. Silinskas and V. M. Maher. Carcinogenesis Laboratory, Michigan State University, East Lansing, MI 48824.

We have demonstrated a dose-dependent increase in the frequency of diploid human cells capable of anchorage independent (AI) growth after treatment with the carcinogen propane sultone, followed by exponential growth to allow full expression of this phenotype. A similar dose-dependent increase in the frequency of 6-thioguanine resistant cells was induced in the population by propane sultone. Procedures such as synchronization of cells before carcinogen treatment and use of special selective medium somewhat enhanced the frequency of AI colonies, but were not essential for this induction. Cells with the AI phenotype were found at a very low frequency in the control cells (background). The data suggest that AI growth is the result of a mutational event. Cells that exhibit the AI phenotype spontaneously or after carcinogen-induced treatment retain it as a permanent characteristic. Colonies of cells demonstrating AI growth (spontaneous or induced) were isolated, grown and injected subcutaneously into athymic mice. Fibrosarcomas formed in these animals at the site of injection. Tumors were removed and the cells put in culture, grown and injected into animals. These cells had a human karyotype and produced tumors of 1 cm diameter within 10 days upon reinjection into athymic mice. Supported by NIH Grant CA 21289.

Neoplastic Progression and Teratocarcinomas

613 PROGRESSION OF PROSTATIC ADENOCARCINOMA IN NB RATS, Neil G. Anderson, Audrey K. Rocco and Leland W.K. Chung, University of Colorado, Boulder, CO 80309

Prostatic adenocarcinoma of dorsolateral lobe origin can be induced in Nb rats by postnatal sex hormone administration (Noble, R.L., Cancer Res., 37:1929, 1977). Utilizing this model, we have examined the progression of prostatic cancer by morphological and biochemical methods. Nb rats were treated with either testosterone proprionate (TP, 0.6 mg/day) or TP plus estradiol diproprionate (E_2P_2, 0.08 mg/day) from weaning until 6-8 months when they were compared to untreated controls. The following were observed: (1) Induction of intense epithelial hyperplasia in the ventral lobe (VP) and selective atrophy and hyperplasia of acini in the dorsal and lateral lobes (DLP) with TP treatment. Extensive fibromuscular proliferation in all lobes with TP + E_2P_2 treatment. (2) The metabolism of testosterone demonstrated a selective 5-fold increase in VP 5α-reductase activity in TP treated animals and a 10-fold reduction in this enzyme activity by TP + E_2P_2 in both the VP and DLP. (3) TP treatment had profound effects on cytosolic androgen receptor content as measured by methyltrienolone (R-1881) binding with no detectable androgen receptor in the DLP. (4) Tissue protein kinase patterns as measured by photoaffinity probe (8-azido-cyclic AMP) revealed new forms of protein kinases following TP or TP + E_2P_2 treatment. Biochemical changes in total protein profile as determined by ^{35}S-methionine incorporation and the rates of synthesis of prostatic α-protein are presently under investigation in this study of important biochemical markers which can be used to assess the progression of prostatic adenocarcinoma. (Supported by CA-27418 and AM-25266).

614 EVIDENCE FOR MULTICLONAL ORIGIN OF DIMETHYLBENZANTHRACENE INDUCED PAPILLOMAS, A.L. Reddy and P.J. Fialkow, VA Medical Center and University of Washington, Seattle, WA

Because of X-chromosome inactivation during embryogenesis, female mice heterozygous at the X-linked phosphoglycerate kinase (PGK) locus for the usual gene (Pgk-1^b) and the variant Pgk-1^a have two populations of cells: one producing type B enzyme, and the other, type A. Tumors with a monoclonal origin exhibit type B or A PGK, whereas those with multicellular origin may show both enzyme types. We used PGK mosaic mice to study skin papillomas induced by 7,12-dimethylbenzanthracene (DMBA) and 12-0-tetradecanoyl phorbol-13-acetate (TPA) dissolved in acetone. Four groups of mice were evaluated: 1) DMBA (200 µg once), 2) TPA (5 µg three times a week), 3) DMBA (200 µg) once + TPA (5 µg) three times a week, 4) DMBA (200 µg) every week. Tumor incidence was very low in Groups 1 and 2. DMBA and TPA (Group 3) induced tumors in 50% of the mice with an average of 2.5 papillomas per mouse. In contrast, the incidence of papillomas in Group 4 (DMBA every week) was 100% and there was also a notable increase in the frequency of papillomas per mouse (10.2). The most striking finding was that 42/90 papillomas in Group 4 showed double-enzyme phenotypes as contrasted with 2/17 in Group 3 ($p < .01$). Thus, these preliminary results suggest that the mechanism of papilloma development differs in these two groups. Repeated application of DMBA alone (Group 4) may induce tumorigenic changes in many cells, producing a large number of papillomas, many of which have a multicellular origin. On the other hand, DMBA treatment once (Group 3) may induce these changes in very few cells and TPA promotes growth of these cells to papillomas. Alternatively, DMBA treatment once may induce tumorigenic changes in many cells, but TPA selects a small number of these cells to grow into tumors. (Supported by the Veterans Administration).

615 A ROLE FOR CHROMOSOME VARIABILITY IN NEOPLASTIC PROGRESSION. B.D. Crawford, R.K. Moyzis M. Melville, and P.O.P. Ts'o, Div. of Biophysics, Johns Hopkins Univ., Baltimore, Md.

If specific karyotypic abnormalities are related causally to neoplasia, analysis of pure populations of neoplastic cells should reveal changes required for tumorigenesis. Studies of BP6T, a clonal subdiploid tumor cell line derived from Syrian hamster embryo (SHE) cells, reveal that extreme karyotypic variability (frequent nondisjunctions and translocations) is a characteristic of these cells. Among 35 banded karyotypes, no two were alike, although the injection of $\leq$10 cells produces tumors in every injected newborn hamster. Fusion of BP6T ($HPRT^-/Oua^r$) and diploid SHE ($HPRT^r/Oua^s$) cells shows initial suppression of the anchorage independence phenotype (AI), a trait correlated with tumorigenicity and a reliable marker of neoplastic conversion in SHE cells (Ca.Res. 39:1504,1979). However, rapid segregation ($1.5x10^{-4}cell^{-1}gen^{-1}$) of AI cells occurs, which approximates the rate of chromosome loss (measured as $HPRT^-$ segregants) in these unstable hybrids. The spontaneous rate of AI conversion in aneuploid SHE cells approximates the rate of single-locus (Oua^r) mutation. The frequency of AI is not increased by point or frameshift mutagens, which induce codominant (Oua^r) but not recessive ($HPRT^-$) mutants of these same subtetraploid cells. These results are consistent with the notion of a recessive gene-dose-dependent nature for the AI phenotype. The measured frequency of nondisjunction in these cells could permit conversion of putative heterozygous preneoplastic cells into neoplastic AI homozygotes at the rate observed. Thus, these studies implicate chromosome variability and random allelic assortment as a potential pathway in neoplastic progression. The effects of a direct perturbation of the karyotype by antimitotic agents are currently under investigation.

616 NEOPLASTIC TRANSFORMATION OF SYRIAN HAMSTER EMBRYO AND ADULT FIBROBLASTS. Sarah A. Bruce and Paul O.P. Ts'o, Division of Biophysics, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland 21205.

To understand the interrelationship among differentiation, the escape from in vitro senescence and in vitro neoplastic transformation, we are studying these phenomena in fibroblast cultures established from 12-day embryos and young (6 mos.) and old (20-26 mos.) adult Syrian hamsters. Adult hamster diploid skin fibroblasts generally senesce after 10-15 population doublings (PD) with no apparent significant difference between cells derived from young versus old hamsters; in contrast embryonic fibroblasts proliferate until 30-60 PD. Embryonic diploid fibroblasts can be neoplastically transformed in vitro by exposure to carcinogens and the acquisition of phenotypes associated with neoplasia occurs progressively over 10-60 or more PD (PNAS 75:3761, 1978). To determine if adult fibroblasts could be neoplastically transformed similarly, and to investigate the effects of the in vitro and in vivo cellular age of the cells on transformation, low passage and high passage skin fibroblast cultures derived from young and old adults have been exposed to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG, 5µM, 2 hrs.) or benzo-(a)pyrene (B(a)P, 10µg/ml, 24 hrs.) passaged in vitro and analyzed for neoplasia related phenotypes. By eight weeks (20-30 PD) after carcinogenic treatment by which time the control cultures had senesced, all treated adult cell cultures contained morphologically distinct cells which continue to proliferate. Anchorage independent growth (cloning in semi-solid agar) which appears late in the transformation of embryonic cells and is highly correlated to tumorigenicity (Ca. Res. 39: 1504, 1979) is first detected in the adult cells 60 PD after treatment. (Supported by NIH Grant #AG 01998).

617 MUTATION RATES OF NORMAL AND CHEMICALLY TRANSFORMED HUMAN SKIN FIBROBLASTS, Eugene Elmore and J. Carl Barrett, NIEHS, Research Triangle Park, NC 27709

Cellular heterogeniety in tumors is well documented. Whether this heterogeniety arises from inherent cellular instability at the genetic or at the epigenetic level is unclear. To test the hypothesis that genetic instability results following chemical transformation of human fibroblasts, we have determined and compared the mutation rates of a normal diploid human skin fibroblast(KD) and a chemically induced transformed line(Hut-11) derived from KD cells. Both lines were generously supplied by Dr. Takeo Kakanuga, NCI. The two genetic loci used in this study were hypoxanthine phosphoribosyl-transferase(HPRT), an X-linked recessive locus, and Na^+/K^+ATPase, an autosomal dominant locus. HPRT mutants were selected by resistance to 6-thioguanine and Na^+/K^+ATPase mutants were selected by resistance to ouabain. Our growth conditions permit routine cloning efficiencies of 70-90% and population doubling times of 16-17 hours with both normal and neoplastic human cells. Mutation rates were determined by Luria-Delbruck fluctuation analysis. The HPRT mutation rates of KD($1.6\text{-}2.1 \times 10^{-6}$/cell/generation) and Hut-11 ($1.0\text{-}1.8 \times 10^{-6}$/cell/generation) cells were not different and compare favorably with previously published rates at this locus. The Na^+/K^+ATPase mutation rates of KD($3.8\text{-}8.5 \times 10^{-7}$/cell/generation) and Hut-11($6\text{-}13 \times 10^{-7}$/cell/generation) cells were also similar. The observed Na^+/K^+ATPase mutation rates are from 5-26 fold higher than previously reported. We feel that our improved growth conditions allow for the growth of previously undetectable ouabain resistant mutants. MNNG induced HPRT mutation frequencies and expression times were also similar. Increased mutation rates do not appear to be a necessary factor in carcinogen induced transformation of human cells.

618 PRENEOPLASTIC PROGRESSION OF SYRIAN HAMSTER CELLS, Leila Diamond, Joseph Sina and Thomas G. O'Brien, The Wistar Institute, Philadelphia, PA 19104

To study the progression of Syrian hamster cells to anchorage independence and tumorigenicity, embryo fibroblasts were initiated with polycyclic aromatic hydrocarbons under conditions of the transformation assay of Berwald and Sachs (J. Natl. Cancer Inst. 35:641, 1965), cell lines were obtained by ring isolation of isolated colonies and followed during long-term cultivation for the development of markers of neoplastic transformation. Twenty independent continuous cell lines were generated from colony isolates maintained in medium containing a phorbol ester tumor promoter; "sister" cultures of 15 of these isolates grown in the absence of promoter senesced by passage 10. At low passage, all clones had a "normal ornithine decarboxylase (ODC) phenotype", with serum and the promoter 12-O-tetradecanoyl-phorbol-13-acetate (TPA) having an additive effect on ODC induction. Prior to acquiring the ability to grow in soft agar, the clones shifted to the "transformed ODC phenotype" of tumorigenic cell lines with the two inducers having a synergistic effect on enzyme activity (Biochem. Biophys. Acta 632:270, 1980). Cell lines established from primary hamster epidermal cell cultures exposed to a carcinogen or SV40 also acquired a transformed ODC phenotype after passage in culture. Thus, altered regulation of polyamine biosynthesis as detected by the response to TPA as an inducer of ODC may be a marker of the preneoplastic state in both fibroblastic and epidermal Syrian hamster cell cultures. (Supported by grants CA21778, CA23413, ES01664 from DHEW).

619 INTERACTION OF POLYOMA VIRUS WITH F9 EMBRYONAL CARCINOMA AND ITS DIFFERENTIATED PROGENY, Katrina T. Trevor and John M. Lehman, University of Colorado Health Sciences Center, Denver, CO 80262.

We are investigating the interaction of the oncogenic virus polyoma with the F9 embryonal carcinoma, a nullipotent cell line which can be induced to differentiate into endodermal derivatives with retinoic acid treatment. Since the undifferentiated stem cells do not express viral early proteins while a limited number of differentiated progeny are capable of complete viral expression, the polyoma genome may serves as a useful probe for understanding gene regulation processes which operate during differentiation. A 5-day exposure to retinoic acid resulted in the morphological differentiation of approximately 60% of the cells. Upon infection after treatment, only 20-25% of the cells became polyoma T- antigen positive within 2 days postinfection. Expression of polyoma was not seen if the cells were infected and subsequently differentiated in the presence of retinoic acid. However, the genome was still present as determined by the Southern technique. Either the genome was permanently suppressed by the formerly undifferentiated cells or the genome was not present in those cells competent for expression. Long-term passage of infected stem cells resulted in low level polyoma T antigen expression as well as virus. In one instance, the virus produced by these cells could infect undifferentiated cells while wild-type polyoma remained suppressed, suggesting the expression of a mutated or altered form of the virus. (This work was supported by grants CA-16030 and CA-15823 from the National Institutes of Health, a grant from the National Science Foundation and a gift from R.J. Reynolds Industries, Inc.)

620 NUCLEOLAR PERSISTENCE IN EMBRYONAL CARCINOMA CELLS, Susan Sheldon, Wendell C. Speers and John M. Lehman, University of Colorado Health Sciences Center, Denver, CO 80262. In most normal cells, the nucleoli disappear during prophase and reappear late in telophase; in a few cell types, nucleoli may persist throughout mitosis. We have examined a series of pluripotent and nullipotent embryonal carcinoma (EC) lines, differentiated lines derived from EC lines, and mouse embryo fibroblasts and bone marrow, as well as human EC, other tumors, and normal tissue in section. Both mouse and human EC cells had persistent nucleoli in 70-85% of mitotic figures. Upon differentiation, either spontaneously or by induction with dimethylacetamide or retinoic acid, the level of nucleolar persistence drops to 15-20%. Differentiated mouse and human cells behave in a similar fashion. The persistent nucleoli contain DNA, and appear to be synthetically active, as they take up ^{3}HUdR, stain with ammoniacal silver, and have the appropriate ultrastructural characteristics. They may be antigenically distinct from the nucleoli of differentiated cells. It appears that loss of nucleolar persistence may correlate well with differentiation of these cells, and may reflect some basic functional differences. (Supported in part by grants CA-16030 and CA-15823 from the National Cancer Institute, a grant from the National Science Foundation, and a gift from R.J. Reynolds Industries, Inc.)

621 CHEMICAL INDUCTION OF TERATOCARCINOMA DIFFERENTIATION IN VITRO: COMPARISON OF RETINOIC ACID AND DIMETHYLACETAMIDE, Wendell C. Speers, University of Colorado Health Sciences Center, Denver, CO 80262.

The murine teratocarcinoma has been used as a model of neoplastic differentiation. The embryonal carcinoma (EC) stem cells of this tumor are highly malignant, but the differentiated progeny are in general benign. EC cells in vitro can be induced to differentiate nearly completely using chemical agents. I have compared the differentiation induced by retinoic acid (RA) and N,N-dimethyl acetamide (DMA) seen with F9 cells and two sublines (A and B) of PCC4azal EC cells. F9 cells responded to RA but not DMA. The A subline EC in monolayer differentiated in different directions with RA and DMA, the latter giving flat epithelial cells with distinct cell borders, the former giving cells morphologically intermediate between fibroblastic and epithelial morphologies. The B subline EC cells responded in a morphologically similar way to both agents with RA giving more rapid and more complete differentiation. B subline cells selected for relative resistance to DMA remained exquisitely sensitive to RA. These results suggest that the mechanisms of RA and DMA induction of differentiation are not the same. (Supported by a gift from R.J. Reynolds Industries, Inc., a grant from The Milheim Foundation for Cancer Research and grant no. CA-15823 from the National Institutes of Health.)

622 REMARKABLE UNIFORMITY OF PREMALIGNANT AND MALIGNANT HEPATOCELLULAR FOCI AFTER SINGLE DOSE INJECTION OF DIETHYLNITROSAMINE (DEN) IN INFANT MICE, S. Goldfarb, S.D. Vesselinovitch, T.D. Pugh, N. Mihailovich, H. Koen and Y. He, University of Wisconsin Medical School, Madison, WI 53706 and University of Chicago, Chicago, IL

Single i.p. injections of DEN (5μg per gram body weight) in 15 day old male C57BL x C3H F_1 mice induced strikingly uniform foci of basophilic hepatocytes with abundant cytoplasm RNA and deficient glucose-6-phosphatase activity. Detailed stereologic and growth kinetic analyses were based on study of 247 profiles of foci in livers of mice (8 mice per group) sacrificed at 10, 20, 28, 36, and 44 weeks post injection, and on serial section reconstructions of 74 foci in two livers of mice sacrificed at 10 and 20 weeks post injection. The foci were first noted at 10 weeks post injection when they were composed of one cell thick hepatic plates. They enlarged over the ensuing 34 weeks, assumed predominantly spherical shapes, and progressed to typical metastasizing trabecular carcinomas (Cancer Res., 38:2003, 1978). All of the foci were probably premalignant or malignant from their inception since (a) they had a 10 to 80 fold increase in ^{3}H-Tdr labeling indices at 20 weeks and later; (b) 20% of them invaded terminal hepatic veins at 20 weeks; (c) they showed a two fold increase in the nuclear to cytoplasmic ratio as early as ten weeks post injection; (d) they had a cumulative volume doubling time of 2.5 weeks, comparable to that of most rapidly growing malignant neoplasms. Conclusion: Because of the strong evidence of clonal origin of these foci, their ease of quantitation, and their rather uniformly spherical shapes, this mouse model is suggested as a potentially useful short term in vivo bioassay of initiators and promoters of hepatocarcinogenesis.

623 SERUM AND TPA STIMULATED INDUCTION OF ORNITHINE DECARBOXYLASE IN C3H/10T1/2 CELLS, Rune Djurhuus and Johan R. Lillehaug, University of Bergen, 5ooo Bergen, Norway.

Several reports have indicated a relationship between the induction of ornithine decarboxylase (ODC) and the tumor promoting activity of 12-O- tetradecanoyl phorbol 13-acetate (TPA). We have studied ODC induction in the C3H/10T1/2 cells in which the 2-stage carcinogenesis process is well established. Our results show that ODC is induced both with serum and TPA. Serum induction results in two peaks of ODC activity, one at 5 and the other at 12 hrs after medium change. TPA induces only the second peak of ODC activity. The ODC induction is inhibited by cycloheximide and actinomycin D at 10 and 1.5 μg/ml respectively. The RNA polymerase II inhibitor, α-amanitin only partly inhibits ODC induction by TPA when added to the cultures 6 hrs after TPA.

The induction of ODC was also tested in cell cultures treated according to the 2-stage carcinogenesis protocol of Mondal *et al.*(1). The results from these experiments suggest that 3-methylcholanthrene initiated cells respond less efficiently with respect to ODC induction than control cultures not receiving 3-methylcholanthrene.

(1) Mondal *et al.*(1976) Cancer Res. 36, 2254-2260.

Supported in part by the Norwegian Council for Sience and Technology.

Somatic Cell Genetics

624 THE USE OF HYBRID CELLS IN AN ANALYSIS OF TUMOUR INVASION AND METASTASIS, Eric Sidebottom, James P. Quigley and Peter B. Armstrong, School of Pathology, University of Oxford, Oxford OX1 3RE

It is now well established that malignancy can be suppressed by the fusion of tumour cells with normal diploid cells such as fibroblasts or lymphocytes. However, segregation in the progeny of the original non-tumorigenic hybrid cells gives rise to some clones of cells which re-express the tumorigenic phenotype. This experimental system therefore provides clones of closely related cells, some tumorigenic, some non-tumorigenic.

Different clones of malignant segregants from hybrid cells produced by fusion of diploid cells with metastatic melanoma cell lines vary considerably in their ability to produce spontaneous metastases after subcutaneous injection in sygeneic mice. We are currently comparing clones of non-tumorigenic and tumorigenic cells and clones of differing metastastic potential for their *in vitro* growth characteristics, their proteolytic activities (plasminogen activating enzymes and collagenase) and their invasive capacity.

Invasion is examined *in vivo* in the chick embryo chorioallantoic membrane and in the hamster cheek pouch, and *in vitro* in organ cultures of isolated tissues such as mouse bladder.

625 A DNA^{ts} MUTANT OF CHINESE HAMSTER OVARY CELLS, Ardythe A. McCracken, School of Life and Health Sciences, Univ. of Delaware, Newark, DE 19711

Studies on macromolecular synthesis in a temperature-sensitive cell cycle mutant, tsC8, of the CHO-K1 cell line, showed a rapid inhibition of DNA synthesis at the nonpermissive temperature (NPT) with little effect on initial levels of RNA and protein synthesis. A progressive increase in RNA content was demonstrated by flow cytometry in temperature arrested tsC8 cells, whereas levels of DNA remained unchanged. Shift up - shift down experiments located the execution point in S phase. The broad functional period, G_1/S to mid S, as defined by these experiments, may be a result of the pleiotropic effect of this mutation. Data from chromatin condensation and premature chromatin condensation analyses of synchronized and nonsynchronized cultures were consistent with a ts step involved in DNA synthesis. Depending on culture conditions, arrest points were observed in either late G_1 or early S. Autoradiographic studies of DNA synthesis after release from hydroxurea block, showed near control level incorporation of ^{3}H-thymidine at the NPT for 2 hours. By 3 hours, DNA synthesis deteriorates and the cells were unable to complete the ongoing cycle. Complementation hybridization demonstrated that tsC8 cells carry a recessive mutation in a gene distinct from those affected in 3 other complementing DNA^{ts} mammalian cell mutants. These mutants will be extremely useful in investigating the molecular biology of the complex coordinated processes of DNA replication.

626 SUPPRESSION OF TUMORIGENICITY IN CYBRIDS: Jerry W. Shay, Gay Lorkowski, and Mike A. Clark, The Univ. of Texas Health Sci. Ctr., Dept., Cell Biol., Dallas, TX. 75235.

The 984 C10 cell line was originally derived from a teratoma that differentiates in cell culture into skeletal muscle. The 984 C10-15 is a stable subclone of this line which does not form myotubes in cell culture and is highly tumorigenic (forms nondifferentiated sarcomas in nude mice). A series of experiments were designed to determine if "normal" cytoplasm obtained from non-tumorigenic cells (AMT-BU-A1) could influence the behavior of undifferentiated, highly tumorigenic 984 C10-15 cells when fused to each other using standard hybridization and genetic selection techniques. The following results were obtained: (1) Numerous subclones of the 984 C10-15 cell line were characterized and all were observed to be tumorigenic while only 3% were capable of differentiating into myotubes in cell culture (9/272); (2) Isolated subclones of the AMT BU A1 cell line were all observed to be non-tumorigenic and all remained undifferentiated in cell culture; (3) The cytoplasmic hybrids (cybrids) clones isolated by fusing AMT BU A1 cytoplasts to 984 C10-15 whole cells yielded the following results. Of 27 cybrid clones isolated and injected into 54 mice, only 3 clones developed nondifferentiated sarcomas in six months, while 58% were observed to form myotubes in cell culture. Interestingly, one of the cybrid clones was both tumorigenic and capable of differentiating in cell culture. These results suggest that cytoplasmic factors in the AMT BU A1 cell can influence the tumorigenic and differentiation capability of the 984 C10-15 cell line but that suppression of tumorigenicity in the cybrids is not always related to the ability of the cybrids to differentiate in cell culture.

627 ANALYSIS OF GENETIC REGULATION OF NUCLEOTIDE SYNTHESIS, David Patterson, Carol Jones, Sharon Graw, and Dale C. Oates, Eleanor Roosevelt Institute for Cancer Research, University of Colorado, Denver, CO 80262 (#335)

Analysis of chromosomal location of the genes of purine nucleotide biosynthesis by somatic cell fusions between various human cells and CHO-K1 cells deficient in purine nucleotide synthesis suggests clustering of the genes coding for the enzymes of this pathway. In particular, two genes, coding for the third [phosphoribosylglycineamide synthetase (GARS)] and sixth [phosphoribosylaminoimidazole synthetase (AIRS)] enzymes of de novo purine synthesis, appear to be located on human chromosome #21 (1). The genes coding for the fourth [phosphoribosylglycineamide formyltransferase (GARFT)] and fifth [phosphoribosylformylglycineamide amidotransferase (FGARAT)] appear to be located on human chromosome 14 (2). Moreover, it is possible to isolate what appear to be single gene mutants which have lost both GARS and AIRS activity in CHO-K1 cells and other mutants which have lost both the first (PRPP amidotransferase) and fifth (FGARAT) activities (3). Mutants defective in each of the individual steps singly can also be isolated. Thus, the third and sixth steps of the pathway and the first and fifth steps of the pathway appear to be coordinately genetically regulated in CHO-K1 cells. Isolation and characterization of the genes coding for these enzymes and isolation and characterization of the enzymatic gene products should allow a full understanding of the genetic and biochemical regulation of this critical metabolic pathway in mammalian cells.

1. Patterson, D., Graw, S., and Jones, C. Proc. Natl. Acad. Sci. USA (in press).
2. Kao, F.T. (1980) J. Cell Biol. 87: 291a.
3. Oates, D.C., Vannais, D., and Patterson, D. (1980) Cell 20: 797.

628 REGULATION OF STAGE SPECIFIC EMBRYONIC ANTIGENS (SSEA), Barbara B. Knowles, and Davor Solter, The Wistar Institute, 36th Street at Spruce, Philadelphia, PA 19104

Cell surface antigenic determinants expressed at specific stages of development of the murine embryo have been identified by the use of monoclonal and conventional antibodies. Several of these antigenic determinants are carbohydrate in nature and may be borne by glycolipid molecules. These antigens are related to common blood group antigens and their precursors.

Regulation of expression of these cell surface molecules is found both in developing embryos and in differentiating teratocarcinoma stem cells. Since the SSEAs are not primary gene products, but the result of the action of glycosyl transferases and/or glycosidases, mechanisms of this regulatory control will be discussed.

629 CHEMICAL CARCINOGENS TRANSFORM BHK CELLS BY INDUCING A RECESSIVE MUTATION
Noel Bouck and G. di Mayorca, Department of Microbiology-Immunology and Cancer Center, Northwestern University, Chicago, IL 60611 and Department of Microbiology, College of Medicine and Dentistry of New Jersey, Newark, N.J.07103

Short treatment of cultured BHK cells with mutagenic carcinogens induces neoplastic transformed clones many of which are restricted by temperature in the expression of their transformed phenotype. The number of transformants induced by increasing doses of carcinogen rises in exact parallel to the number of mutants to 6-thioguanine resistance and ouabain resistance which are induced in the same experiment. The phenotype of transformation is recessive. Somatic cell hybrids between the normal parental cell line and recently derived transformants are of normal morphology and fail to clone in soft agar. Control hybridizations pairing the same normal cell with a BHK line transformed by polyoma virus consistently produces transformed hybrids which clone well in agar. An explanation of the origin of these chemically induced transformants which is consistent with the above data and with information available on in vitro transformation of other rodent cell lines is the following: Chemical carcinogen treatment produces a mutation (often temperature sensitive in BHK) in a gene which happens to be hemizigous in this easily transformed line and whose product is required for normal anchorage dependent growth.

630 MOLECULAR BASIS OF GENETIC DEFECTS IN HUMAN GLOBIN GENES. Monica Shander, Susan Vande Woude, Nicholas Proudfoot and Tom Maniatis. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Each member of the human α-like and β-like globin gene families has been isolated and extensively characterized using molecular cloning procedures (see (1) for review). A comparative analysis of different globin gene sequences has identified putative transcription and mRNA processing signals, provided interesting information regarding globin gene evolution (2) and demonstrated the existence of human globin pseudogenes (3). As one means of correlating sequence organization and gene function we previously demonstrated the feasibility of studying the in vitro transcription of human globin genes (4). Our results to date are: 1) The δ-globin gene which is expressed less efficiently than the β-globin gene in adult erythroid cells is also poorly transcribed in vitro; 2) The α-globin pseudogene Ψα1 is transcribed in vitro but less efficiently than the normal α-globin gene; 3) β-globin genes were isolated from the DNA of seven different individuals with β^{0}-thalassemia, a genetic disease characterized by the complete absence of β-globin polypeptide. All of these genes are transcribed in vitro with the same efficiency as that of the normal β-globin gene. Comparison of the nucleotide sequence of one of the β^{0}-globin genes with the sequence of the normal β-globin gene (5) revealed the presence of a G to A transition within the GT sequence located at the 5' end of the large intervening sequence. It is possible that a transcript from this β^{0} gene would not be spliced. In vivo experiments are in progress to test this possibility, using a SV40 vector system.
(1) Maniatis, T., Fritsch, E. F., Lauer, J., and Lawn, R. M. (1980) Ann. Rev. Genetics 14, 145-178. (2) Efstratiadis et al., (1980) Cell 21, 653-668. (3) Proudfoot, N. J. and Maniatis, T. (1980) Cell 21, 537-544. (4) Proudfoot, N. J., Shander, M. H. M., Manley, J. L., Gefter, M. L., and Maniatis, T. (1980) Science 209, 1329-1336. (5) Lawn, R. M., Efstratiadis, A., O'Connell, C. O., and Maniatis, T. (1980) Cell 21, 647-651.

631 WHICH GENES ARE INVOLVED IN CHEMICAL TRANSFORMATION OF C3H/10T1/2 AND MMCE CELLS IN VITRO?, Ulf R. Rapp and Ed Birkenmeier, National Institutes of Health, National Cancer Institute, Frederick Cancer Research Center, Fort Detrick, Frederick, Maryland 21701.

Two cell systems were used for chemical transformation in vitro, the fibroblast cell line C3H/10T1/2 and the epithelial mouse cell line MMCE. Carcinogens used included MCA (methylcholanthrene), ENU (ethylnitrosourea) and ENU plus the promoter TPA. Transformed cells were cloned through soft agar before further analysis. The DNAs from several of these clones have been tested by others for transforming activity (Shih et al, PNAS 76, 5714, (1979), Wigler, unpublished data) and were found to be positive. From these experiments it appeared that in these cells transformation was due to the action of presumably a single gene. We had tested previously by superinfection rescue and BUdR activation experiments whether such a gene may be an endogenous type C virus or a virus-cell recombinant. However, neither competent nor defective transforming viral genomes were readily rescuable or inducable from these cells (Rapp et al., Virology 65, 392, 1975). Recently these experiments were extended as follows: 1. Transforming viral genomes were isolated from chemically transformed cells chronically infected with endogenous MuLV (Rapp and Todaro, PNAS 77, 624, 1980). We have molecularly cloned these genomes and examined their structure and biological activity. 2. Transformed cells were then examined for expression of these new oncogenic viral genomes as well as for a series of known type C virus coded tumor genes (Bister, Rapp and Duesberg, unpublished data). The results of these experiments will be presented.

632 DELINIATION OF THE PROMOTER OF A STRUCTURAL GENE, Steven L. McKnight, Carnegie Institution of Washington, Department of Embryology, Baltimore, Md. 21210.

The Herpes Simplex Virus (HSV) thymidine kinase (tk) gene is expressed in the form of enzymatically functional tk when microinjected into Xenopus laevis oocyte nuclei. This reaction is sensitive to low levels of α-amanitin, indicating that tk gene expression in frog oocytes is mediated by RNA polymerase form II. Moreover, the 5' terminus of tk mRNA synthesized by frog oocytes is identical to that of tk mRNA produced in HSV infected mamalian cells. I have used the coupled transcription/translation system of the frog oocyte as an assay to deliniate the promoter of the tk gene. A systematic library of extragenic deletion mutants was constructed by the Exo III/S1 procedure (Sakonju et al, 1980). These mutants lack varying segments of the DNA sequences flanking the 5' terminus of the tk gene. The precise end point of each deletion mutant was defined by DNA sequencing. Subsequently, each mutant was microinjected into frog oocytes. The responses of oocyte assays to microinjection of various deletion mutants are as follows. Isolates harboring 109 or more nucleotides of 5' flanking DNA elicit a "wild type" level of accurate tk mRNA when injected into oocytes. Isolates with deletion end points ranging from 95 to 32 nucleotides upstream from the tk gene produce tk mRNA having the proper 5' terminus, yet at 1/10 to 1/20 the "wild type" level of response. Mutants that maintain less than 24 nucleotides of 5' flanking DNA do not support the synthesis of authentic tk mRNA. It is concluded that the structural gene promoter consists of two distinct elements. One functional component, located at least 95 nucleotides upstream from the transcribed portion of the gene, is required for quantitative expression by RNA polymerase II. The other component, located at least 25 nucleotides from the gene, is required for an accurate transcriptional start.

633 DNA MEDIATED TRANSFORMATION OF HUMAN EPITHELIAL AND FIBROBLAST CELLS
John Hiscott, Mark Steinberg, and Vittorio Defendi, NYU Medical Center New York,NY 10016.

Human cell strains of epithelial and fibroblastic origin have been transformed by DNA-mediated gene transfer using a variety of purified viral genes and DNA segments. These fragments include a DNA segment which contains both early viral and murine sequences that was cloned from SV40 tsA58 transformed mouse cells. The human diploid fibroblasts (FS-4) maintain multiple copies of tsA DNA fragments, largely in a plasmid state, as analyzed by blot hybridization and the persistence of these DNA fragments can be modulated by incubation at 40°C and/or by continued passage of the transfected cells in the presence of homologous interferon. Whereas the fibroblastic cells tend to become senescent after an extended lifespan, DNA transfected human epithelial cells can be selected with low efficiency as SV40 transformants which maintain integrated and free viral genes.

634 A VARIANT OF S49 MOUSE LYMPHOMA CELLS WITH ENHANCED MUTAGENIC RESPONSES TO ALKYLATING AGENTS, Mark A. MacInnes and Philip Coffino, U. of California, San Francisco,CA 94143

Early events in chemical carcinogenesis may include alkylation induced mutations and chromosomal aberrations. To study the genetic controls governing alkylation mutagenesis we have characterized a variant(211.1.2) of S49 mouse lymphoma cells with a 2.5 to 3.0 fold enhanced mutability relative to the parental strain(24.3.2) for the alkylating agents ethyl methanesulfonate and N-methyl-N'-nitro-N-nitrosoguanidine. Enhanced dose-responses with these agents are seen both for mutations to 6-thioguanine resistance and ouabain resistance in 211.1.2. This variant does not exhibit a significantly increased dose-dependent cytotoxicity than parental cells with either agent. The mutation responses of 211.1.2 are apparently specific to simple alkylations since U.V.light, 2-aminopurine(an adenine analog), and ICR-191(a quinacrine mustard) do not induce greater mutability or cytotoxicity in the variant than in parental cells. We are now testing the hypothesis that 211.1.2 has a defect in alkylation specific DNA repair that leads to enhanced mutagenesis but not increased cytotoxicity.

Cell Growth Factors and Mitogenesis

635 MELITTIN, MONOVALENT IONS, AND MITOGENESIS, Thomas D. Gelehrter and Enrique Rozengurt, Univ. of Michigan, Ann Arbor, MI, and Imperial Cancer Research Fund, London, U.K.

Melittin is an amphipathic polypeptide of bee venom which binds to phospholipids and increases membrane permeability. Because increased Na flux may mediate the proliferative response of quiescent cells to growth factors, we asked whether melittin could trigger ion fluxes and DNA synthesis in quiescent Swiss 3T3 cells. Melittin at 350 ng/ml causes a rapid increase in ouabain-sensitive ^{86}Rb uptake, a measure of Na-K pump activity. The toxin enhances pump activity by increasing Na entry into the cells. Other early events associated with the proliferative response are not elicited by the toxin. At concentrations which promote ion fluxes, melittin, acting synergistically with insulin, is a potent mitogen, stimulating DNA synthesis in quiescent mouse cells in serum-free medium.

Because alterations in phospholipid methylation and/or deacylation may be the regulatory signals for mitogenesis, and because melittin is known to activate phospholipase-A_2 (PLase) we examined the effects of melittin and other mitogens on phospholipid deacylation in quiescent 3T3 cells. At concentrations which stimulate monovalent ion fluxes and DNA synthesis, melittin has little effect on PLase activity. The Na ionophore monensin increases pump activity without affecting PLase activity; whereas the calcium ionophore A23187 stimulates PLase activity but not the Na-K pump. Finally, vasopressin and insulin potently stimulate ion fluxes and DNA synthesis but have no effect on PLase activity. Thus, phospholipid deacylation appears not to be required for the activation of early (ion fluxes) or late (DNA synthesis) biochemical events associated with the proliferative response of quiescent fibroblasts.

636 ALTERATIONS OF INTERMEDIATE FILAMENTS IN MOUSE EPITHELIAL CELLS AFTER VIRAL TRANSFORMATION. J. Keski-Oja, V.-P. Lehto, T. Vartio and I. Virtanen. Depts of Virology and of Pathology, University of Helsinki, Helsinki, Finland.

Mouse embryo epithelial cells, MMC-E, were transformed by Moloney MSV or MuLV, and clones of transformed cells were isolated from soft agar. Cytoskeletons of the cells were studied by indirect immunofluorescence technique using affinity-purified antibodies and by SDS-PAGE. Polypeptide analysis of the cells by SDS-PAGE showed similar amounts of keratin polypeptides in all cells but the amount of vimentin was distinctly increased after transformation. MMC-E cells showed fine fibrillar filaments of vimentin-type, but keratin was located only to distinct phase-dense perinuclear granules. MMC-E cells transformed by MoMSV or MuLV showed distinctly different cytoskeletal organization. The amount of vimentin seemed to be greatly increased in both of these virus-transformed cell clones. On the other hand, the MuLV-transformed cells but not the MoMSV-transformed cells exhibited also bright fibrillar keratin-specific fluorescence in all cells. In addition, both the nontransformed MMC-E cells and their MoMSV or MuLV-transformed counterparts showed dense fibrillar arrays of microtubules. The results indicate that transformation of mouse epithelial cells by MoMSV or MuLV can induce different changes in the cytoskeleton of these cells.

637 PROLIFERATIVE PROPERTIES OF HUMAN T LYMPHOCYTE PROGENY INITIALLY ACTIVATED BY CONCANAVALIN A, Allen J. Norin, Montefiore Hosp., Albert Einstein College of Medicine, New York, N.Y. 10467

Immortal lymphoblastoid cell lines can be established by culturing lymphocytes from patients with lympho-proliferative diseases. These cell lines grow indefinitely in standard culture medium supplemented with a small amount of serum. Normal human lymphocytes can be grown in standard media however proliferation requires the addition of an exogenous mitogenic agent to the culture. We have investigated the properties of concanavalin A activated human T lymphocytes and their progeny. After about 30 hrs of incubation some cells no longer required mitogen and thus were committed to an initial round of DNA replication at 42 to 48 hrs. Because primary lymphocyte cultures are heterogeneous the proliferative behavior of the progeny resulting from the first stimulation was monitored by determining what proportion of DNA molecules that were labelled with ^{14}CdT in a initial S phase achieved a hybrid density after incubation BrdU. I found that the progeny of the initial division required an additional mitogenic signal in order to undergo a second round of replication and that the second division occurred 6 to 12 hrs sooner than the first one. Thus, in order to maintain normal lymphocytes in a proliferative mode they required exposure to exogenous mitogens or possibly to mitogenic agents excreted by activated cells. ^{14}CdT pulse-chase experiments demonstrated that the viability of previously activated but quiescent cells required the presence of a mitogenic agent. Carcinogens may, in part, activate a gene(s) for an endogenous mitogenic factor that eliminates the requirement for an exogenous agent. This work was supported by an NIH grant Ca 22088.

638 LOSS OF EGF-DEPENDENT PHOSPHORYLATION OF A 170,000 Mr MEMBRANE GLYCOPROTEIN AND MALIGNANT TRANSFORMATION. J.A. Fernandez-Pol, VA Medical Center and St. Louis University, St. Louis, Missouri 63125

Addition of epidermal growth factor (EGF) in vitro to membranes prepared from normal rat kidney (NRK) cells stimulated ^{32}P incorporation from [γ-^{32}P] ATP into many specific membrane proteins. One of these membrane components, an intrinsic membrane glycoprotein of Mr 170K, which was weakly phosphorylated in the basal state, was primarily and specifically affected by EGF. Incubation of membranes from Kirsten sarcoma virus-transformed NRK (K-NRK) cells with [γ-^{32}P] ATP demonstrated at least 10 components whose phosphorylation was enhanced by EGF. In K-NRK membranes we found a specific loss of the EGF-dependent phosphorylation of the 170K Mr protein. In revertants of K-NRK cells, which resemble NRK cells in their morphology and growth characteristics, the EGF-dependent phosphorylation of the 170K Mr protein was restored to control levels. Other experiments demonstrated that: (a) NRK cells have high levels of EGF receptors; (b) K-NRK cells exhibited a marked reduction in EGF receptors; (c) EGF receptors were restored to control levels in revertant cells. Further studies with a series of clonal revertant cell lines which differ in their degree of tumorogenicity demonstrated that the loss of both EGF receptors and EGF-dependent phosphorylation of the 170K Mr protein was quantitatively associated with the ability of the revertant clones to proliferate in semi solid agar. The data suggest that the 170K Mr protein is a component of the receptor for EGF which is a substrate of the phosphorylation reaction. The results are compatible with the supposition that K-NRK membranes lose the 170K Mr phosphoprotein as a result of malignant transformation.

639 EFFECTS OF TUMOR PROMOTING PHORBOL ESTERS (PE), SACCHARIN (S), AND CYCLAMATE (C) ON THE BINDING OF INSULIN (IGF), EPIDERMAL GROWTH FACTOR (EGF), AND ^{3}H-PHORBOL DIBUTYRATE (PDBU) - TUMOR PROMOTION INVOLVING A HORMONE RESPONSE CONTROL UNIT (HRCU) AND ITS THRESHOLD-SENSITIZING ACTION. L. S. Lee, General Electric Corporate Research and Development, Schenectady, New York 12301

Our recent studies in the past two years have shown that: (1) PE inhibited EGF binding, (2) C and S inhibited EGF binding, (3) the most potent phorbol ester, 12-0-tetradecanoyl phorbol 13-acetate (TPA), did not inhibit IGF binding, (4) PE binds to a specific receptor, (5) S and C inhibited IGF binding, (6) S, C, and TPA did not inhibit concanavalin A binding, and (7) S and C did not inhibit TPA binding. These inhibitions were observed with TPA at concentrations of 10^{-7} to 10^{-9} M or S and C at concentrations of 10^{-1} to 10^{-3} M in the assay of various monolayer cultures of cells derived from human, dog, rat, hamster, and mouse.

We have proposed that there exists a HRCU which regulates the hormone response signals and, if stimulated for a prolonged period and in excess of a threshold value, may mediate the promoting process, as in the case of EGF as a tumor promoter. Depending on the interaction, TPA receptor complexes, S and C inhibit EGF binding through HRCU, while S and C can also inhibit IGF binding through HRCU.

PDBU binding assay showed that mezerein has much less affinity with the PE receptor than TPA. Since mezerein is not tumor-promoting alone, but promotes after TPA treatment, we have proposed that tumor promoter may function by gradually lowering the threshold level of HRCU stimulation. When this level in an initiated cell is below the normal physiological level, the cell is locked in the transformed state and the cancer cells are said to be at a permanently promoted state in any growth conditions.

640 BENZO(A)PYRENE AND OTHER INDUCERS OF CYTOCHROME P_1-450 INHIBIT BINDING OF EPIDERMAL GROWTH FACTOR (EGF) TO CELL SURFACE RECEPTORS, Vesna Ivanovic and I. Bernard Weinstein, Columbia University, New York, N.Y. 10032.

In the present study the binding of ^{125}I-labelled EGF was utilized to monitor possible cell surface effects of polycyclic aromatic hydrocarbon carcinogens. Exposure of confluent 10T½ mouse fibroblasts to 1μM benzo(a)pyrene (BP) led to a time-dependent decrease of EGF binding. By 24 hours, EGF binding was only about 5% that of control cultures. In contrast, BP-7,8 diol-9,10-oxide did not significantly alter EGF binding, indicating that the inhibition by BP was not simply due to DNA damage. Several other hydrophobic chemicals were tested at 1-4μM for inhibition of EGF binding. Progesterone, 17β-estradiol and cholesterol had very little effect. Nor was significant inhibition observed with phenobarbital, pp'DDT, hexachlorobenzene or pregnenolone-16α-carbonitrile, compounds which induce forms of P-450 that are distinct from P_1-450. On the other hand, the known inducers of P_1-450 were very effective inhibitors of EGF binding in the following order of potency: dimethylbenz(a)anthracene > BP > benz(a)anthracene > 3-methylcholanthrene > β-naphthoflavone. Our results correlate with published data on the apparent affinities of these compounds for a cytosolic binding protein, the Ah receptor. It has been proposed that the induction of cytochrome P_1-450 and several other enzymes is mediated by this receptor. We postulate, therefore, that inhibition of EGF binding, and possibly other cell surface effects, are components of the pleiotropic response triggered by occupancy of the Ah receptor. The possible relevance of these findings to the tumor promoting activities of certain complete carcinogens will be discussed. (Supported by Grant CA-21111 from the NCI).

641 CO-ISOLATION FROM HEPATIC TISSUE OF STIMULATORS AND INHIBITORS OF IN VIVO LIVER REGENERATION. Loren Pickart, W.H. Goodwin, William Burgua and J.C. Houck, Virginia Mason Research Center, Seattle, WA 98101

The liver contains undefined factors of 2,000 to 12,000 D which inhibit in vivo hepatic replication (hepatic chalone). In an effort to isolate these factors, bovine liver was blended with a Polytron homogenizer, extracted with 63% ethanol, then precipitates obtained at 63, 70, 87 and 95% ethanol concentration, plus 95% ethanol and equivolume acetone, by centrifugation at 20,000 G in a continuous-flow zonal rotor. Precipitates were dissolved in H_2O and filtered through an Amicon UM-10, then retarded on a UM-2 filter. The final supernate from the centrifugation was flash-evaporated and processed similarly. Filtrates were fractionated on a Sephadex LH-20 column (2.5 x 100 cm), then lyophilized for testing.

Powders from LH-20 column fractions were injected I.P. into mice 15 h after partial (50%) hepatectomy (HX), ^{3}H-thymidine injected at 19 h, and liver tissue obtained at 20.5 h. Activity was assayed as thymidine incorporation into DNA. Most bioactivity was in material which precipitated from 95% ethanol after addition of equivolume acetone. When tested at 1 mg/mouse, LH-20 fractions of K_{av} 0.15 and 0.67 stimulated, while K_{av} 0.51 inhibited DNA synthesis (Control (Sham-operated) = 1, HX = 7.8, HX + K_{av} 0.15 = 13.4, HX + K_{av} 0.51 = 4.8, HX + K_{av} 0.67 = 10.4). Autoradiography gave similar results.

These results demonstrate that procedures that isolate hepatic inhibitors also isolate stimulatory factors. The co-isolation of bioactivities suggests that in vivo stimulators and inhibitors of hepatic replication have similar physiochemical characteristics and may possess similar structures. This work supported by USPHS Grants CA 27129 and RR 05588.

642 ANALYSIS OF HUMAN EGF RECEPTOR USING ANTISERA SPECIFIC FOR A HUMAN 165K PROTEIN Cathleen R. Carlin, David P. Aden, Barbara B. Knowles, Wistar Institute, Philadelphia, PA 19104

A 165K cell surface glycoprotein coded for by the short arm of human chromosome 7 has previously been characterized in our laboratory. The glycoprotein was originally defined using antisera made by injecting mice with man-mouse somatic cell hybrids (cl 21, cl 36) that contained chromosome 7 as their only human component. Others have reported that (1) the receptor for EGF (EGFR) is a 165-185K cell surface protein and (2) expression of EGFR in somatic cell hybrids is associated with the presence of human chromosome 7.

Our results indicate that the human EGFR and the 7-coded 165K protein are related. In competitive inhibition studies, we find that preincubation of human cells (WI-38, LNSV, A431) with αcl 21 antiserum blocks binding of ^{125}I-EGF and that the ability to bind EGF is restored at high antiserum dilution. The same dilution of α cl 21 that completely blocks EGF binding by human cells inhibits 40 to 50% of EGF binding to cl 21 or cl 36 cells, indicating that the hybrid cells express a mixed population of human and mouse EGFR's; again, ability to bind EGF is completely restored at high antiserum dilution. The antiserum has no effect on EGF binding by an immunoselected hybrid clone that has segregated the short arm of the human 7. Further experiments designed to determine whether the 165K protein and human EGFR are identical and whether binding of αcl 21 antiserum by cells mimics binding of EGF will be discussed.

643 CALMODULIN (CaM) PLAYS AN IMPORTANT ROLE IN G_1/S TRANSITION, J.G. Chafouleas, W.E. Bolton, A.E. Boyd, III, and A.R. Means, Departments of Cell Biology and Medicine, Baylor College of Medicine, Houston, TX 77030.

It is now well established that CaM mediates most if not all of the Ca^{2+} regulated events in non-muscle cells. Since it is likely that the regulation mediated by this multifunctional protein is directly related to its effective concentration at any given time, the following study was undertaken to evaluate the regulation of CaM during the cell cycle and to correlate this to cell cycle specific events. The cell cycle parameters of CHO-K_1 cells were ascertained by the pulse labeled mitosis procedure. Cells were synchronized in M without the use of drugs by the mitotic shake procedure. Cells with mitotic indices of 93-98% were released into G_1 and samples removed at regular intervals for determination of CaM levels by RIA. Progression through the cell cycle and verification of synchrony was monitored by determining the percent of the cells labeled at each point with a 10 min. ^{3}H TdR pulse as well as the percent mitotic cells. CaM levels during M are 1.5×10^{-13} gr/cell and in early G_1, following cell division, this amount is reduced to 7.8×10^{-13} gr/cell. The CaM levels begin to increase during late G_1 and reach the maximum levels observed in M by early S. This pattern is observed from cells having G_1 periods of 2 or 5 hr demonstrating that CaM is synthesized entirely during late G_1 or early S. In order to evaluate whether CaM was important in the G_1/S transition, cells were treated with the anti-CaM drug W13 or its inactive homolog W12. While W12 had no effect, W13 caused a reduction in total cell number after 24 hrs. Analysis of these treated cells by flow cytometry revealed a dramatic reduction in S phase cells with a concomitant build up of G_1 cells. These data suggest CaM plays an important role in progression of cells from G_1 into S.

644 MODULATION OF GROWTH FACTOR REQUIREMENTS OF BP-3T3 AND 3T3 CELLS, A.P. Whipple, W.J. Pledger, C.D. Stiles, and C.D. Scher. Harvard Medical School, Boston, MA. University of North Carolina, Chapel Hill, NC.

The addition of platelet derived growth factor (PDGF) or fibroblast growth factor (FGF) to density arrested BALB/c-3T3 cells induces them to become competent to synthesize DNA. Plasma and nutrients regulate the entry of competent cells into the S phase. Pretreatment of cells affects their sensitivity to the growth factors. BALB/c-3T3 cells arrested in 0.5% plasma are more sensitive to the PDGF or plasma components of serum than cells arrested in 10% plasma. These cells do not synthesize DNA after transfer to fresh medium lacking serum. A clonal isolate of benzo(a)pyrene transformed BALB/c-3T3 BP-3T3 cells grows equally well in plasma or serum supplemented medium demonstrating that they do not require PDGF. BP-3T3 cells maintained in medium with 10% serum or plasma become growth arrested at confluence with a G_1 DNA content. Transfer to fresh medium lacking serum induces DNA synthesis in 50% of the cells. Addition of an optimal concentration of plasma allows 80% of the cells to enter the S phase. Purified PDGF or FGF has no effect. When BP-3T3 cells become growth arrested in 0.5% plasma, their responsiveness to nutrients and growth factors changes. The cells can now be stimulated by competence factors (PDGF or FGF) or plasma, but do not respond to the addition of nutrients alone. BP-3T3 cells have lost the requirement for competence factors, but under certain conditions will respond to them. Unlike 3T3 cells, BP-3T3 cells arrested in 10% plasma will synthesize DNA after transfer to fresh serum-free medium.

645 CYTOPLASMIC MEDIATOR OF PLATELET-DERIVED GROWTH FACTOR ACTION, J.C.Smith*, C.D.Stiles*, W.J. Pledger†, and C.D. Scher*, *Sidney Farber Cancer Institute, Boston, MA 02115, †University of North Carolina, Chapel Hill, NC 27514.

Density-arrested BALB/c-3T3 cells treated with platelet derived growth factor (PDGF) become 'competent' to replicate DNA. They enter S phase following a lag time of 12 hrs when incubated in medium containing plasma. When PDGF-treated competent cells are fused to untreated cells, the resulting heterokaryons become competent to replicate DNA. Cytoplasts derived from PDGF-treated cells are also able to render recipient cells competent. When donor cells are treated with the inhibitors of RNA synthesis actinomycin D or 5, 6-dichloro-β-ribofuranosylbenzimidazole (DRB) during exposure to PDGF, they are unable to transfer competence; this suggests that transfer of competence is due to a 'second message' rather than PDGF itself and that the production of this second message required RNA synthesis. Cycloheximide does not prevent competence transfer indicating that this second message can be RNA.

Analysis of cytoplasmic proteins of PDGF-treated cells demonstrates the preferential synthesis of several proteins with molecular weights ranging from 29K to 70K daltons within 40-90 min. Both the rate of synthesis of one of these proteins (pI) and the percentage of cells induced to become competent are related to the concentrations of PDGF. Pituitary FGF has a similar activity, but EGF, insulin or plasma do not. Treatment of cells with actinomycin D or DRB prevents the synthesis of pI in response to PDGF. The data suggest that the synthesis of cytoplasmic protein(s) like pI is necessary for BALB/c-3T3 cells to become competent.

646 *GROWTH FACTOR RESPONSIVENESS OF G_1 ARRESTED PREDIFFERENTIATED 3T3 T PROADIPOCYTES IS* DISTINCT FROM DENSITY-INHIBITED G_1 3T3 T CELLS, Dagne L. Florine, Bryan J. Hoerl, Han-Seung Yoon and Robert E. Scott, Section of Experimental Pathology, Depts. of Anatomic Pathology & Cell Biology, Mayo Clinic/Foundation, Rochester, MN 55901

We have recently established that, prior to differentiation, growth arrest of 3T3 T proadipocytes occurs at a specific state in the G_1 phase of the cell cycle. This arrest state, designated G_D, is distinct from the G_1 growth arrest states induced by density-dependent growth inhibition (G_S) and nutrient deprivation (G_N). The critical observation that serves as a basis for this conclusion is that G_D arrested cells can be induced to express an adipocyte phenotype without cell proliferation but G_S and G_N arrested 3T3 T proadipocytes cannot. We now report that there are also differences in the mitogenic responsiveness of G_D, G_S and G_N arrested cells to growth factors and mitogens. In particular, G_D arrested cells are not responsive to the proliferative effect of many mitogens which stimulate the growth of G_S and G_N arrested cells. G_D arrested cells can, however, be stimulated to proliferate by certain biological fluids, especially cyst fluid derived from 3T3-induced mouse tumors, by certain chemicals and by replating G_D arrested cells at low density following trypsinization. Studies are now in progress to identify and characterize the active mitogenic cyst fluid fraction and to determine the mechanism of action of the various mitogens for G_D arrested cells. It is our hypothesis that growth arrest of 3T3 T proadipocytes prior to differentiation in the G_D arrest state is associated with a change in the expression of mitogen receptors on the cell surface or with an uncoupling of the metabolic events that result from mitogen-receptor interaction.
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647 DETECTION OF "TRANSFORMING GROWTH FACTOR" IN MOUSE EMBRYOS, Jacqueline A. Proper and Harold L. Moses, Dept. of Cell Biology, Mayo Clinic/Foundation, Rochester, MN 55901
"Transforming growth factor(s)" (TGF) capable of inducing nontransformed indicator cells to form colonies in soft agar has been isolated from conditioned medium of transformed cells growing in culture, as well as from tumor cells both *in vitro* and *in vivo* utilizing an acid/ethanol extraction procedure.[1] TGF's, including sarcoma growth factor, compete for binding to the epidermal growth factor (EGF) receptor, but do not cross react with EGF antibodies.[2] Extracts of 17 day mouse embryos have been shown to have more EGF receptor competing activity than could be accounted for by EGF detected by radioimmunoassay,[3] suggesting the possibility of embryonic production of a TGF-like growth factor. An acid/ethanol extraction of 17 day fetal mice yielded a growth factor capable of inducing nontransformed mouse AKR-2B and rat NRK cells to form progressively growing colonies in soft agar. This "fetal growth factor(s)" has an apparent molecular weight of 12,000 to 14,000 and possesses the ability to stimulate DNA synthesis and confer a transformed morphology on nontransformed cells *in vitro*. The activity of this factor(s) is destroyed by treatment with either trypsin or dithiothreitol, but is unaffected by heat (56° for 30 min or 100° for 3 min), indicating that the activity is due to a heat stable polypeptide with disulfide bonds. The presence of this growth factor in 17 day mouse embryos suggests that both fetal development and neoplastic transformation may be affected by similar polypeptides.
[1]Roberts, et al., *Proc. Natl. Acad. Sci. USA 77:*3494-3498, 1980.
[2]DeLarco, J.E. and Todaro, G.J. *J. Cell. Physiol. 102:*267-277, 1980.
[3]Nexø, et al., *Proc. Natl. Acad. Sci. USA 77:*2782-2785, 1980.

648 FETAL BOVINE SERUM CONTAINS "TRANSFORMING GROWTH FACTOR", Chris L. Bjornson and Harold L. Moses, Dept. of Cell Biology, Mayo Clinic/Foundation, Rochester, MN 55901
"Transforming growth factor" (TGF), which causes nontransformed anchorage-dependent cells to form colonies in soft agar, has been isolated from murine sarcoma virus-transformed cells and other tumor cells *in vitro* and *in vivo* (Roberts et al., *Proc. Natl. Acad. Sci. USA 77:*6, 1980). Studies in our laboratory indicate the production of TGF by the chemically transformed AKR-MCA and C3H/MCA-58 mouse cells and from 17 day mouse embryos. The detection of TGF in fetal tissue suggested the possibility that fetal serum may contain similar factors. To test this possibility, fetal bovine serum (FBS) was precipitated with ammonium sulfate according to the procedure described for fetuin purification (Pedersen, *J. Phys. Colloid Chem. 51:*164, 1947). An acetic acid extract of the precipitate was subjected to gel filtration and the activity in different fractions was determined by their ability to stimulate growth in soft agar. Peak activity was found in the 10-12,000 molecular weight range which is markedly different from the 47,000 molecular weight of fetuin. The activity was destroyed by treatment with trypsin or dithiothreitol but not by heat (56° for 30 min or 100° for 3 min) suggesting that the activity is due to a heat stable polypeptide with disulfide bonds. The partially purified factor from FBS caused stimulation of DNA synthesis and morphologic transformation in AKR-2B cells and induced colony formation in soft agar by nontransformed mouse AKR-2B and rat NRK cells. Preliminary studies indicate that calf serum has substantially less TGF-like activity than FBS. The presence of TGF-like substances in FBS could be of significance in supporting growth of certain cells in culture.

649 SURFACE DIFFERENCES BETWEEN MITOTIC AND NON-MITOTIC THYMOCYTES, Janet G. Salisbury, Dept. Biochem. St George's Hosp. Med. School, London SW17 ORE,UK.
The initiation of cell division has been widely studied (e.g. mitogenesis) but the converse i.e. switching-off of cell division has gained little attention. However, an important difference between normal and tumour cells is that the latter apparently are unable to switch-off cell division in response to appropriate stimuli. This may reflect a change in regulatory cell surface components. Thymocytes have been used to study this problem since they can be broadly classified into two populations. The larger cells (10-20% of the total) are rapidly dividing and are precursors for the majority of smaller non-dividing cells. The two populations have been separated for both rat and mouse thymocytes (Salisbury et al., 1979, J. Biochem. Biophys. Methods, 1, 341) and the surface proteins have been compared by labelling with ^{125}I or NaB^3H_4. Marked differences have been observed by SDS-PAGE in proteins of 30-60,000 mol. wt. (Salisbury and Graham, Biochem. J. 192, in press). Thymomas have been induced in mice by a single injection with 80mg/kg MNUa and the surface proteins of the thymoma cells are currently being studied using the same methods.
The role of cellular proteins in viral carcinogenesis has recently received much attention e.g. a 54K membrane-bound protein ("middle T" antigen) has been shown to be increased on tranformation by SV40 (Levine et al., 1980, Eur. J. Cell Biol. 22 526). The same protein is also elevated in other transformed cells, including those induced by chemicals. Further identification of proteins which may be involved in control of normal cell division, and altered on transformation may eventually shed more light on the mechanisms involved in carcinogenesis.

650 TYPE II REGULATORY SUBUNITS OF CYCLIC AMP-DEPENDENT PROTEIN KINASES CHANGE DURING POSTNATAL AND NEOPLASTIC MOUSE LUNG DEVELOPMENT. Alvin M. Malkinson and Martin S. Butley, School of Pharmacy, University of Colorado, Boulder, CO 80309.

A natural ligand which can inhibit cell proliferation, and can also reverse the neoplastic phenotype and promote tumor regression is cyclic AMP (cAMP). A decreased responsiveness to alterations in intracellular cAMP concentrations may increase the proliferative rate of neoplastic tissue and also help maintain the neoplastic state. Since the biological effects of cAMP are mediated by an activation of protein kinase enzymes, we studied the interactions of cAMP with one of its intracellular receptors, the regulatory subunit of the Type II kinase isozyme, R_{II}. These interactions included the affinity of R_{II} for the photoaffinity analog, 8-azido-(^{32}P)-cAMP, and the modulatory effects of cAMP on the autophosphorylation of R_{II}. Both parameters were found to vary with the developmental stage and the neoplastic state of the lung. Normal adult lung R_{II} has two 8-azido-(^{32}P)-cAMP binding sites, one which saturates at 125 nM and one which saturates at 800 nM. The R_{II} from urethane-induced pulmonary adenoma appears to have only the low affinity site, while R_{II} from neonatal lung shows greatly reduced binding to both sites. These changes in ligand binding characteristics correlated with changes in the cAMP dependence of R_{II} autophosphorylation. In adult lung, cAMP can either stimulate or inhibit endogenous phosphorylation of R_{II}, depending on the divalent cation present during the reaction. In the tumor, cAMP has little stimulatory effect but is strongly inhibitory. The amount of P^{32}-phosphate that can be incorporated into neonatal R_{II} is very slight with either cation.

651 ROLE OF POLYAMINE LEVELS IN GROWTH REGULATION OF CHINESE HAMSTER V79 AND MOUSE LYMPHOMA S49 CELLS, Lisa McConlogue, Russ Fields, Philip Coffino, University of California at San Francisco, San Francisco, CA 94143

An early and striking response of many cell systems to tumor promoters, mitogens, and transformation is the increase in levels of polyamines and induction of the rate limiting enzyme for their synthesis, ornithine decarboxylase (ODC). We have utilized a specific inhibitor of ODC, α-difluoromethylornithine (DFMO) to study the role of ODC and polyamine levels in normal cellular proliferation of mouse lymphoma S49 and Chinese hamster V79 cells. We also compare DFMO arrest to the arrest of S49 cells by dibutyryl cyclic AMP (Bt_2cAMP).

DFMO treatment of exponentially growing V79 and S49 cells results in inhibition of ODC activity, cessation of cellular proliferation and decrease in intracellular levels of putrescine and spermidine. Flow microfluorometry analysis shows that S49 arrest is in G_1 whereas V79 arrest shows no cell cycle specificity. All effects are reversed by addition of 10 (S49) or 100 (V79) μM putrescine.

S49 cells are also arrested in G_1 by Bt_2cAMP. However, although ODC activity is extinguished, addition of exogenous polyamines (up to 1mM) will neither prevent nor reverse the block. Furthermore, spermidine levels (expressed as nanomole per ml of culture) remain constant in Bt_2cAMP arrested cells whereas DFMO arrest results in spermidine pools decreasing with a half life of 6 to 7 hours.

These data indicate that inhibition of ODC and depletion of polyamines result in cessation of cellular proliferation but that regulation of ODC activity is not causally related to arrest of S49 cells by Bt_2cAMP.

652 GENETICS OF RECEPTORS FOR BIOACTIVE POLYPEPTIDES: A VARIANT OF SWISS/3T3 FIBROBLASTS RESISTANT TO A CYTOTOXIC INSULIN ACCUMULATES LYSOSOME-LIKE VESICLES. W.K. Miskimins, W.R. Ferris and N. Shimizu, Dept. of Cell. & Dev. Biol., U. of Arizona, Tucson, AZ 85721.

As a means for investigating the mechanism of action of polypeptide hormones and growth factors we are utilizing the selection and characterization of genetic variants. In this effort we have constructed selective agents with high specificity to surface receptors by crosslinking the cytotoxic fragment A of diphtheria toxin (DTa) to insulin or epidermal growth factor (EGF)(Miskimins & Shimizu, B.B.R.C.,91:143, 1979; Shimizu et al., FEBS Let., 118:274, 1980). Subsequently, six variants of Swiss/3T3 mouse fibroblasts were isolated as resistant to insulin-DTa hybrid molecules. All of these variants proved to have greatly reduced or no insulin binding capacity as well as altered morphological and growth characteristics, yet, retaining the capacity to bind EGF. Strikingly, one of these variants, CI-3, displays a massive accumulation of membranous vesicles in its cytoplasm. By electron microscopy these vesicles resemble, but are not identical to, lysosomes. These vesicles also appear to fluoresce bright orange after treatment of the viable cells with acridine orange. However, the specific activity of lysosomal enzymes (acid phosphatase, β-glucuronidase and β-galactosidase) is rather depressed in this variant, indicating the unique nature of these vesicles. Experiments are underway to determine the origin and biochemical properties of these vesicles, possible role in maturation and processing of hormone receptors and relation to the CI-3's loss of insulin binding capacity. (supported by Grants from NIH, GM24375 and ACS, JFRA9).

653 THE CYTOSKELETON AND CELL FORM IN NORMAL AND NEOPLASTIC CELLS IN VITRO, B.R. Brinkley, A.R. Means, R.L. Pardue, L.J. Wible, R.M. Cailleau‡ S.M. Cox, R. Brown* and R. Arlinghaus‡ Baylor College of Medicine and *The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

The distribution and organization of cytoplasmic microtubules and actin-containing microfilaments was investigated in a variety of normal and neoplastic cells in vitro including normal human mammary epithelium, 13 human breast carcinoma cell lines, mouse 3T3 and SV3T3 cells and normal rat kidney (NRK) cells infected with a temperature-sensitive transformation mutant of Moloney murine sarcoma virus. Using tubulin antibodies and indirect immunofluorescence, three distinct cell phenotypes were recognized in human breast carcinoma cells. One phenotype was similar to normal mammary epithelium while the remaining two displayed an altered cytoplasmic microtubule complex (CMTC) and fewer actin cables. Viral transformed cells displayed greatly altered cytoskeletons. The onset of cytoskeletal changes in NRK cells corresponded to the synthesis of a virus specific 85,000 dalton polyprotein containing a transformation-specific 25,000 dalton peptide. Studies using a lysed-cell system suggest that altered microtubule assembly is not due to alteration in the tubulin molecule but suppressed activity at the microtubule organizing center.

654 EPIDERMAL GROWTH FACTOR RECEPTORS IN CHEMICALLY TRANSFORMED CELLS, Robert A. Robinson and Harold L. Moses, Departments of Cell Biology and Anatomic Pathology, Mayo Clinic/Foundation, Rochester, MN 55901

The 3-methylcholanthrene transformed AKR-MCA cells arrest growth in G_1 at saturation density due to depletion of low molecular weight nutrients. On the other hand the parent nontransformed AKR-2B cells are capable of a low serum (growth factor deficiency) G_0 arrest 12 hours from S and a G_1 nutrient deficiency arrest point (NAP) 6 hours from S similar to that of the AKR-MCA cells. At G_0 the AKR-2B cells demonstrate 10^5 epidermal growth factor (EGF) receptors per cell and are stimulated to enter S by addition of EGF. At the G_1 NAP both the AKR-2B and AKR-MCA cells bind little EGF and are unresponsive to EGF addition. The present study was undertaken to determine if chemical transformation is related to cell cycle specific changes in EGF binding. Treatment of G_1 arrested AKR-MCA cells with various enzymes and compounds known to dissociate receptor-ligand complexes or unmask binding sites failed to increase EGF binding. Previous studies in this laboratory have shown that readdition of low molecular weight nutrients to nutrient arrested nontransformed AKR-2B cells resulted in increased ^{125}I-EGF binding. We have found that readdition of low molecular weight nutrients to G_1 arrested AKR-MCA cells similarly resulted in a 5 to 10 fold increase in ^{125}I-EGF binding. This increase in binding was prevented by Actinomycin D or cycloheximide. Similar results were obtained with the C3H/MCA-58 cell line which is a chemically transformed derivative of the C3H/10T½ cells. The data indicate that the chemically transformed cells show a significant reduction of EGF receptors only when at the G_1 nutrient deficiency arrest point.