Institute of Environmental Medicine

RESEARCH IN ENVIRONMENTAL HEALTH SCIENCES

Seventeenth Annual Report of Progress

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RESEARCH

IN

ENVIRONMENTAL HEALTH SCIENCES

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I. INTRODUCTION

This report describes the ongoing activities of the New York University Institute of Environmental Medicine. It also serves as the Institute's annual report to the National Institute of Environmental Health Sciences (NIEHS), which provides substantial support for the operation of the Institute through Center Grant ES 00260. This grant, along with a smaller award from the National Cancer Institute, furnishes core support for the professional staff and for some of the centralized facilities of the Institute. Operational support for the various research activities derives principally from federally-funded grants and contracts and, to a lesser degree, from awards made by private foundations and industry.

The Institute is directed by Dr. Arthur C. Upton, former director of the National Cancer Institute. Dr. Upton recently succeeded Dr. Norton Nelson, who served with distinction as Director for 25 years, during which time the Institute attained worldwide recognition for leadership in the environmental area.

The Institute, which has been in existence for over 30 years, had its origin at the New York University Medical Center in Manhattan. Much of the work of the Institute continues to be centered in New York, where the vast resources of the city provide an unparalleled atmosphere for teaching and research. It became apparent, however, that certain environmental studies could better be carried out in surroundings that were less congested and where environmental interferences could be reduced to a minimum. Accordingly, in 1962 some of the Institute's activities were transferred to Sterling Forest, a research and residential park situated approximately 50 miles northwest of New York City. Today the work continues at both locations, where the unique features of each are maximally exploited.

The mission of the Institute is two-fold: to train graduate and medical students for productive careers in research and other service in the environmental area, and to conduct research directed toward the identification and control of environmental health hazards. Current studies encompass a broad spectrum of interests, ranging from investigation of the biomedical and ecological effects of low level ionizing radiation and chemicals to exploration of the mechanisms and pathogenesis of cancer and other diseases.

Implementation of the Institute's mission is carried out by a faculty of 40 and a supporting professional staff of 135, many of whom hold advanced degrees. There are also approximately 90 graduate students and a number of post-doctoral fellows who are supported, in part, by training grants from NIEHS, NCI, and DOE. Students may matriculate either in the Graduate School of Arts and Science, in one of the basic sciences, or in a special interdepartmental Program in Environmental Health Sciences. In each case, thesis research is supervised by a faculty member of the Institute. A specialized Master's degree program in occupational hygiene is also available.

For convenience, this report has been organized into two broad parts: the first part (Section II) presents an overview of the activities of the Institute, and the remaining sections provide more detailed descriptions. The latter sections also contain bibliographic references for further in-depth reading.

II. SUMMARY OF RESEARCH AND TEACHING PROGRAMS

A. RESEARCH

1. Research in Toxicology

In environmental toxicology there are three major goals: (a) the appraisal of the ability of a particular agent or combination to produce adverse health effects, (b) the estimation of the risk that the effect will actually be produced in man under a given set of circumstances, and (c) the development of an understanding of the mechanisms involved in order that preventive action might be taken. Work in progress during the past year has dealt with the characterization and quantification of agents to which man might be exposed, and the study of the effects of these agents on animals in terms of impaired behavioral, physiological, and biochemical function. The toxicology program involves a group of investigators representing a number of disciplines and using a wide variety of experimental techniques.

Palmes and Gunnison (Laboratory of Toxicology) continued to work on the passive personal samplers for gases originally developed at this Institute. The NO_2 samplers are now being used by many groups, and there is a need for much discussion with users concerning the theory, assembly, exposure, and analysis of the devices. A theoretical aspect of the NO_2 sampler investigated empirically was the effect of pressure on sampling rate; according to Fick's First Law, the two factors should be completely independent. Although departures from theory were found to be very minor where sampling on earth is concerned, they might become considerable if sampling by diffusional techniques is done at great altitudes. A graduate student was awarded a Master's degree on the basis of these experiments.

A new phase in the investigation of diffusional samplers was initiated involving the measurement of concentrations of a test gas at several points along the length of the diffusional path. The purpose of this approach is to make it possible to measure both efficiency and capacity of various sorbents without developing a final analytical procedure for the trapped gas.

Evans, Daniel and Teal (Laboratory of Toxicology) have investigated discriminative function related to age in animals and in humans in which normal human adults and children have volunteered to perform the computer-controlled test of accuracy of discriminating visual shapes and time duration. By determining the degree of correlation between test results from normal animals and humans, the validity of using animal tests for predicting the behavior of humans has been established.

Evans, Daniel and Teal (Laboratory of Toxicology) have compared the neurotoxicity of acrylamide in pigeons and in mice. The battery of tests used included food intake, visual and motor coordination, hind

limb grip strength, and complex discriminative behavior. All of the behavioral tests documented early effects of acrylamide before signs such as ataxia and loss of body weight were observed. Mice and pigeons were affected at similar doses (from 20 to 60 mg/kg in subacute exposures, and 100 to 200 mg/kg in acute exposure), indicating the validity of these two animal models for studies in neurotoxicology, and of the pigeon as an alternative to the rodent.

Evans, Teal and Palmes (Laboratory of Toxicology) have begun a study of new assays for neurotoxicity induced by organic solvents. Mice, pigeons and macaque monkeys have been chosen as models for human symptoms of appetite change and sleep disturbances which result from sub-chronic inhalation of benzene, toluene, trichloroethylene or hexane. Some of the new assays being investigated are sufficiently streamlined to permit their use in routine toxicity screening. Other assays are more complex and time consuming. The general issue is whether the abbreviated methods differ significantly from their more complex counterparts in terms of their ability to demonstrate potential occupational health hazards.

Gunnison, Dulak, L. and Palmes (Laboratory of Toxicology) have continued their studies of the metabolism and toxicity of sulfite and sulfur dioxide in mammals. In these investigations, a sulfite oxidase-deficient rat model has been exploited as a means of generating relatively high systemic sulfite concentrations without the incorporation of unrealistic amounts of sulfite salts into the diet. Sulfite metabolism was characterized in these and in normal rats by monitoring the concentrations of sulfite and of two of its metabolites (S-sulfonate compounds and thiosulfate) in the body tissues and fluids.

Subchronic toxicity experiments of approximately 2 months duration using female rats less than 5 months of age revealed a low (2-3%) incidence of mammary adenocarcinoma in sulfite oxidase-deficient individuals compared to a 0% incidence in individuals with normal amounts of this enzyme. There was no suggestion of a dose-response relationship with respect to systemic sulfite exposure. In related experiments, it was demonstrated that anemia and thiamine deficiency, which had been produced previously in sulfite feeding studies, were attributable to the interaction of sulfite with constituents of the diet and not to systemic sulfite exposure. As part of the toxicological evaluation of sulfite, its potential teratogenicity was investigated in sulfite oxidase-deficient rats. No treatment-related effects were observed in the incidences of external or soft tissue morphological abnormalities, or in the number of resorptions, litter size, or mean pup weight.

A study of the reaction of sulfite with the protein disulfide bonds in rabbit plasma has recently culminated in the identification of albumin and plasma fibronectin as the sole proteins containing reactive sites. This underscores the selective nature of disulfide bond sulfitolysis. Further investigation of the sulfitolysis of plasma fibronectin revealed

no changes in several physicochemical and physiological properties of this altered protein.

Gunnison, Dulak, L. and Palmes (Laboratory of Toxicology) have continued their studies of the tissue distribution in rabbits of S-sulfonate metabolites resulting from the inhalation of SO_2 in the range of the current occupational standard. Rabbits exposed to 10 ppm SO_2 for up to 72 continuous hours showed an absence of S-sulfonates in the aortic walls despite the fact that the aortic walls have a high concentration of sulfite-reactive sites. This indicates that little or no sulfite was transported to tissues removed from the site of SO_2 absorption. Significant S-sulfonate formation was observed in the tracheal walls of exposed animals, and within 3 hours after the beginning of SO_2 inhalation an equilibrium between SO_2 and tracheal S-sulfonate concentration was attained. Continuous exposure for longer than 24 hours, however, resulted in further tracheal S-sulfonate formation which was attributed to increased mucus production. Mucus collected from the nasal passages and by extraction of tracheal wall tissue of unexposed rabbits was shown to contain reactive sites for S-sulfonate formation in the high molecular weight glycoproteins as well as in smaller, serum-like proteins.

In cooperation with Lippmann and Schlesinger (Laboratory for Aerosol and Inhalation Research), the metabolism in rabbits of a monodisperse ferric sulfite aerosol is being investigated. To insure penetration of the aerosol into the upper airways, rabbits are made to inhale the aerosol through a cuffed endotracheal tube and are then sacrificed for tissue analysis. Although these studies have just begun, preliminary results indicate little or no formation of S-sulfonate metabolites either in the tracheal walls or in the posterior lobes of the lung.

Schlesinger and Lippmann (Laboratory for Aerosol and Inhalation Research) are continuing an investigation of the effects of inhaled sulfuric acid and Fe(III)-S(IV) aerosols upon particle deposition, airway resistance and mucociliary clearance, using the rabbit as an animal model. The study involves acute and chronic exposures to H_2SO_4, and acute exposure to S(IV) aerosol.

A new aerosol delivery system has been developed to permit exposures of the animals to test materials by mouth breathing since this allows more effective penetration of the aerosol into the lungs. Reproducible bronchial clearance curves are being obtained in the animals prior to initiation of exposure to the sulfur oxide aerosols. A system which allows the simultaneous monitoring of clearance with subsequent automated data analysis in up to ten rabbits has also been developed for use in chronic exposures of rabbits to sulfuric acid mist.

A system is being set up for measurement of levels of exhaled ammonia in rabbits, to determine if endogenous NH_3 is a mediator of the response to inhaled sulfuric acid aerosols. Measurement of ammonia during acid exposure tests will be performed by a chemiluminescence analyzer.

Lippmann, Leikauf, Spektor (Laboratory for Aerosol and Inhalation Research) and Albert (Laboratory of Experimental Medicine) have extended their studies of the effects of 1 hour H_2SO_4 aerosol exposures on mucociliary clearance to include 10 volunteers with past histories and/or current symptoms of asthma and/or bronchitis. Previous studies with 10 healthy nonsmokers found significant alterations in mucociliary clearance following 1 hour inhalation exposures to H_2SO_4 at concentrations as low as 100 $\mu g/m^3$. In these studies, the volunteer subjects inhaled a 7.5 μm (MMAD) Fe_2O_3 aerosol tagged with Tc-99m, and external measurements of retention of the tagged particles were made within a low-background facility in order to monitor mucociliary particle clearance.

For the asthma/bronchitis group, and for an additional group of 10 nonsmokers, a smaller particle size, 4.1 μm (MMAD), was used. The smaller particles compensate for the proximal shifts expected in regional deposition for people with chronic airway bronchoconstriction. This allows a comparison between the asthma/bronchitis group and healthy nonsmokers based on both the same aerosol size and on similar deposition patterns.

Studies have been completed with 9 subjects having asthma and/or bronchitis. As a group, they were not more responsive to H_2SO_4 exposures in terms of bronchial mucociliary clearance changes than the first group of healthy nonsmokers. However, exposure to 1000 $\mu g/m^3$ H_2SO_4 produced consistent changes in respiratory mechanics in 6 of the 9 subjects. The group averages, based on 9 subjects, of $FEV_{1.0}$, MMEF, and V_{25} were depressed by 17, 24, and 32 percent respectively. However, these effects were not statistically significant ($0.05 < p < 0.1$). In tests with 4.1 μm Fe_2O_3 on 3 healthy nonsmokers, the H_2SO_4 exposures produced changes in mucociliary clearance similar to those seen with the 7.5 μm particles, and there were no apparent changes in their respiratory mechanical functions.

Jaeger and Cote (Laboratory of Toxicology) have continued their research on the characterization of the biological action of acrylonitrile (ACN). This work, which is being done in collaboration with Drs. Szabo and Silver (Harvard Medical School and Peter Bent Brigham Hospital Departments of Pathology), has shown that diet is an important factor in the acute toxicity of ACN.

A diet high in fat, whether complete in lipotropic factors (i.e. methionine, choline and niacin) or not is associated with an increased lethality (i.e. lower LD_{50}) in both male and female rats. This diet was also found to be associated with longer hexobarbital sleeping time and elevated brain and liver glutathione content. The increased toxicity observed after oral dosing was not seen in male rats after inhalation exposure, suggesting that hepatic metabolism is a critical factor in acute toxicity.

A sex difference in metabolism was suggested by the finding that females were resistant to the toxic effects of inhaled ACN. Covalent binding of radio-labeled acrylonitrile to macromolecules was greater in rats on the high fat diet. Since mixed function oxidase was diminished while glutathione content measured as non-protein sulfhydryl content was elevated, previous work would have suggested that these conditions would be associated with decreased toxicity and diminished binding rather than increased acute effects. Covalent binding studies using ^{14}C-ACN given orally to both males and females showed that blood contained the highest specific radioactivity due to ^{14}C-ACN or its metabolites while liver contained the greatest total amount of radioactivity as a function of the administered dose. Binding to trichloroacetic acid insoluble elements in the brain, while substantial, did not explain the increased sensitivity of animals on a high fat diet to ACN.

In other studies on ACN, Cote and Jaeger have examined the possibility that ACN may act via the metabolic release of cyanide. Pretreatment of rats with a non-lethal concentration of ACN protected against a subsequent LD_{75} administration of this agent. There was, however, no protection against the lethal effects of cyanide at the LD_{75}. In a second set of experiments, males were shown to be slightly more resistant than females to the effects of cyanide, while females were much more resistant than males to the acute toxic effects of ACN.

To explore the differences in resistance further, the effects of enzyme induction by a polychlorinated biphenyl (Aroclor 1254) were studied. There appeared to be a slight increase in susceptibility to ACN in enzyme-induced males compared to control males. However, enzyme-induced females were clearly more susceptible than control females. In fact, the susceptibility of enzyme-induced females was similar to that of control males. In the above studies, the predominant signs of ACN toxicity observed shortly before death were apparently due to increased sympathetic nervous system activity. In other experiments, animals were pretreated with the anticholinergic agent, atropine. This pretreatment eliminated many of the ACN toxic signs but caused no alteration in the lethality of ACN.

Goldstein and Witz (Laboratory for Experimental Medicine) continued studies evaluating the mechanism by which exposure to ozone or nitrogen dioxide potentiates bacterial infections in the lung. Alveolar macrophages from rats exposed to either of these pollutants have a dose-dependent decrease in ability to generate superoxide anion radical, which is involved in the bacteriocidal activity of phagocytic cells.

A related study of the effect of cadmium in this system demonstrated that relatively low concentrations produce an increase in superoxide anion radical formation and the related burst of oxygen consumption in stimulated phagocytes. This might explain the observed *in vivo* pro-oxidant effects of this relatively inert metallic pollutant. Studies have also continued with Troll to test the hypothesis that free radicals

and related active species derived from activated phagocytic cells may be involved in tumor promotion. The studies have shown that protease inhibitors and retinoids, which are known inhibitors of tumor promotion, also inhibit the production of superoxide anion radical by stimulated phagocytic cells. In addition, a study of a series of phorbol esters has revealed a good correlation between tumor promoting potency and the ability of the compounds to stimulate active radical production by human polymorphonuclear leukocytes.

Continuing studies on the hematological effects of environmental agents have focused on the development of techniques to measure cell membrane fluidity. Preliminary results indicate that ozone, various metals, and lipid peroxide decomposition products are capable of altering red cell membrane fluidity, as measured by fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene. Benzene studies in progress, in addition to those described under Inhalation Toxicology, have focused on the synthesis of pure trans,trans-muconaldehyde for study of its possible role as a metabolite responsible for benzene hematotoxicity.

Troll, Goldstein, Witz and Stone (Laboratory of Biochemical Toxicology) have studied the formation of superoxide anions by the cell membrane in response to PMA, as an important factor in tumor promotion. The oxygen radical response, which is the biological weapon employed by leukocytes to kill bacteria, appears to occur on the cell membrane of a variety of cells. Known inhibitors of tumor promotion -protease inhibitors, dexamethasone, and retinoids - blocked the production of free oxygen radicals.

Troll (Laboratory of Biochemical Toxicology) has carried out studies of hydrogen peroxide release by fertilized sea urchin eggs. Hydrogen peroxide formation was blocked by protease inhibitors and retinoids, while hydrogen peroxide produced in the absence of inhibitors was destroyed by catalase. The addition of protease inhibitors, retinoids or catalase to the sea urchin egg system resulted in polyspermy. Vitamin A was toxic to sperm, causing their inactivation, but superoxide dismutase counteracted the toxicity of vitamin A, suggesting that superoxide anions may be involved in vitamin A toxicity.

Penn, Burns and Albert (Vascular Toxicology Program, Laboratory of Experimental Medicine) have continued studies on the patterns of growth and development of spontaneous arterial lesions in the cockerel, and on atherogenic responses which follow chronic exposure to chemical carcinogens.

In one series of experiments, untreated young cockerels and others treated with 7,12-dimethylbenz(a)anthracene (DMBA) were examined at 4-week intervals for evidence of aortic lesion (plaque) development. The prevalence, size, and proliferation of any plaques observed were noted. The data showed that there were no significant differences in the numbers of plaques found in the two groups of animals. Plaques seen in the

DMBA-treated animals, however, were 10 times the size of those of the controls, and the pattern of proliferation of these larger plaques was different. In a repeat of the experiment in which the ultrastructural details of the plaques were studied by scanning electron microscopy, no significant differences were seen between the two groups of animals, either in plaque surface characteristics, or in cross-sectional appearance.

In other studies, the uptake of DMBA by the smooth muscle cells of the cockerel aorta was examined *in vivo*. The uptake of the compound appears to be a complex process which requires at least two stages, the first of which is compartmentalization in the extracellular space.

2. Research in Chemical Carcinogenesis

Understanding the disease called cancer is exceedingly difficult since it is not a single disease. It is a large family of diseases which show a wide range of structural and behavioral similarities and deviations from what is regarded as "normal". The problem is further compounded by the fact that even our understanding of the normal cell is relatively rudimentary. In a very real sense, therefore, the comparisons that are made between the aberrant cancer cell and the normal cell are comparisons between unknowns. As a consequence, the research described below involves basic biological investigation, as well as detailed study of environmental factors such as carcinogens and other factors which initiate or modulate the carcinogenic state.

Van Duuren, Solomon and Segal (Laboratory of Organic Chemistry and Carcinogenesis) have used gas chromatographic/computerized mass spectral techniques to elucidate the structure of compounds important in the carcinogenic process. Carcinogen-modified nucleosides and DNA bases were identified using electron impact and isobutane chemical ionization mass spectrometry, and various derivatization methods were developed to increase the stability of thermally labelled materials. O-acylated adducts formed from the interaction of the carcinogen dimethylcarbamyl chloride with deoxyguanosine and deoxythymidine were also characterized. The structure of 3-(2-carboxyethyl)cystosine was established, and the compound was identified as a product of the *in vitro* reaction of the mouse skin carcinogen, β-propiolactone, with calf thymus DNA.

Van Duuren and Banerjee (Laboratory of Organic Chemistry and Carcinogenesis) are continuing investigations into the covalent interaction of trichloroethylene (TCE), an extensively used organic solvent, with cellular macromolecules. Earlier work demonstrated that an active intermediate(s) of TCE bound covalently to hepatic microsomal proteins of $B6C3F_1$ mice and Osborne-Mendel rats and to exogenous DNA. Studies also indicate a differences in the binding of TCE to macromolecules that were dependent upon the species, strain, sex, age, and organ of the animal. The binding of TCE to proteins and DNA from $B6C3F_1$ mice, during incubation with microsomes from the mice, was significantly higher than the corresponding binding to macromolecules from Osborne-Mendel rats. This is interesting because the mice are susceptible to TCE-induced liver tumorigenesis, while the rats are not.

Microsomal proteins from the lung, stomach, and kidney of $B6C3F_1$ mice also metabolized TCE. The binding of TCE to liver proteins from Sprague Dawley rats was higher than that to proteins from Osborne-Mendel and Fischer 344 rats. DNA and protein binding were enhanced by prior *in vivo* phenobarbital administration, and they were inhibited by SKF-525A, 7,8-benzoflavone and GSH in the *in vitro* binding system. The effects of 1,2-epoxy-3,3,3-trichloropropane on the binding of TCE to macromolecules were also examined.

Van Duuren, Bannerjee and Kline (Laboratory of Organic Chemistry and Carcinogenesis) have continued studies on the mode of action and the structure-activity relationships of halogenated hydrocarbons.

1,2-Dibromoethane (DBE), a pesticide and a known potent animal carcinogen, has been shown to bind covalently to stomach and liver microsomal proteins and to salmon sperm DNA. The results suggest that metabolic activation of DBE is required for the covalent binding to macromolecules.

The microsome-mediated covalent binding of DBE to chromatin isolated from forestomach and liver of $B6C3F_1$ mice has been investigated. DBE, which is a stomach carcinogen in $B6C3F_1$ mice, but not a liver carcinogen, interacted less with microsomal and chromatin proteins of mouse stomach than with proteins of liver. The binding of DBE to microsomal and chromatin protein, as well as to chromatin DNA, was higher in liver than in forestomach. DBE bound equally to proteins isolated either from microsomes or from chromatin of forestomach and liver. On the other hand, the binding of DBE to chromatin DNA of forestomach and liver was significantly higher than the binding to salmon sperm DNA. The results of studies on the binding of ^{14}C-DBE to fractionated chromatin indicated that DBE interacted more with nonhistone chromosomal proteins than with histones.

In order to determine whether the covalent binding of the carcinogen 1,2-dichloroethane (DCE) to macromolecules is dependent upon metabolic activation, microsomes and cytosol from lungs of $B6C3F_1$ mice and Osborne-Mendel rats were incubated with ^{14}C-DCE and salmon sperm DNA. DCE bound covalently to microsomal protein and DNA only in the presence of microsomes, while cytosol produced insignificant metabolic activation. The interaction of DCE with DNA was enhanced following pretreatment of the animals with phenobarbital and 3-methylcholanthrene, but glutathione reduced the binding. The binding of DCE to macromolecules from $B6C3F_1$ mice was significantly higher than the binding to macromolecules from Osborne-Mendel rats. Furthermore, DCE interacted significantly more with macromolecules in the presence of microsomes from lung than from liver of $B6C3F_1$ mice.

Studies have continued on the characterization of the adducts formed from the *in vitro* reaction of chromatin DNA with DBE in the presence of rat liver microsomes. In order to characterize potential adducts, bromoacetaldehyde, an *in vitro* metabolite of DBE, was reacted with the four deoxynucleosides over a range of pH's, and all major products were isolated and characterized.

Agarwal, Van Duuren (Laboratory of Organic Chemistry and Carcinogenesis) and Kneip (Laboratory of Environmental Studies) have continued their studies on the detection of adducts produced by the trapping of epoxides with nucleophiles by examining the reaction of epoxides with the nucleophile 4-nitrothiophenol. The study has shown that the mono-

and diepoxides of aliphatic and aromatic hydrocarbons undergo facile nucleophilic opening by 4-nitrothiophenol to yield the expected 1, 2 adducts clearly and in good yields. This method is highly suitable for trapping epoxides since the adducts produced yield distinctive peaks in chemical ionization mass spectroscopy, when single ion monitoring is used.

Epoxides are important because they are often mutagenic and carcinogenic, and they are important industrial chemicals. Epoxides are also likely products of photooxidation and, therefore, they may be present in the atmosphere.

Van Duuren, Agarwal and Kline (Laboratory of Organic Chemistry and Carcinogenesis) have initiated a new study of carcinogens produced in the disinfection of drinking water supplies. Such carcinogens can be produced in the chlorination or ozonization of humic materials in natural water supplies.

In these studies, commercial humic materials are being evaluated by a series of chemical and physical methods. A series of chronic bioassay studies using Sencar mice has also been initiated which will establish the suitability of Sencar mice for chronic bioassays with chlorinated humic and fulvic acids.

Van Duuren, Kline and Melchionne (Laboratory of Organic Chemistry and Carcinogenesis) have studied the bacterial mutagenicity, transformation in cell culture, and carcinogenicity of a series of chloroalkene epoxides. The mutagenicity studies were carried out in collaboration with Dr. H. Rozenkranz of New York Medical College, and the cell culture studies were carried out in collaboration with Dr. J. DiPaolo of the National Cancer Institute. The expoxides were: *cis-* and *trans*-1-chloropropene oxide (*c*- and *t*-CPO), *cis-* and *trans*-1,3-dichloropropene oxide (*c*- and *t*-DCPO), trichloroethylene oxide (TCEO), and perchloroethylene oxide (PCEO). Mutagenicity was assayed without microsomal activation in *S. typhimurium* TA1535 and in *E. coli* WP2 strains, and genotoxicity was assayed in *E. coli* pol A^+/A^-. With the exception of TCEO, all compounds were mutagenic in *S. typhimurium*, with *c*- and *t*-DCPO exhibiting the greatest potency. Generally, similar results were obtained with *E. Coli* and pol A^+/A^-, but in these systems, TCEO did show appreciable activity.

Transforming ability was determined with a quantitative Syrian hamster embryo model. All epoxides produced transformation at least at one concentration, with PCEO, *t*-CPO and *t*-DCPO being the most effective. Toxicity, as indicated by reduced cloning efficiency, was similar to that seen in bacteria. The compounds were also tested for carcinogenicity by skin application and by subcutaneous injection in female ICR/Ha mice. The four test compounds *c*-CPO, *t*-CPO, *c*-DCPO, and *t*-DCPO produced significant incidences of local tumors by both routes of administration. TCEO and PCEO did not cause significant incidences by either route, however.

Van Duuren, Segal and Solomon (Laboratory of Organic Chemistry and Carcinogenesis) have been studying the *in vitro* interactions between both the potent direct-acting acylating rodent carcinogen dimethylcarbamyl chloride (DMCC) and the less potent carcinogen diethylcarbamyl chloride (DECC), with calf thymus DNA. Reaction between DMCC and 2'-deoxyguanosine (dGuo) in H_2O at pH 7.0-7.5 and 37°C gave a small amount of blue fluorescent compound. Based on UV, NMR, and fluorescence spectra and electron impact and isobutane chemical ionization mass spectra, the structure assigned to the fluorescent compound was the O^6-acyl derivative of dGuo, O^6-dimethylcarbamyldeoxyguanosine (O^6-DMCdGuo). The diethyl analog, O^6-diethylcarbamyldeoxyguanosine (O^6-DECdGuo) resulted from reaction of DECC with dGuo. *In vitro* reaction of DMCC with calf thymus DNA followed by enzyme hydrolysis to nucleosides resulted in the identification of O^6-DMCdGuo by high-pressure liquid chromatography. Reaction in H_2O between either O^6-DMCdGuo or O^6-DECdGuo with dimethylamine resulted in a novel nucleophilic aromatic substitution to give 2-amino-N^6-dimethyldeoxyadenosine. The reaction did not occur with NH_3 or diethylamine. The results of these studies are significant because of the association which has been established between O^6-alkylation of guanine in DNA and mutagenesis and carcinogenesis.

Segal and Solomon (Laboratory of Organic Chemistry and Carcinogenesis) have been studying the sites of interaction in DNA of the mouse skin carcinogen β-propiolactone (BPL). The new adduct 3-(2-carboxyethyl)-cytosine (3-CEC) was isolated following *in vitro* reaction of the carcinogen with calf thymus DNA, and the structure of 3-CEC was confirmed by synthesis from BPL and dCyd. Reaction of BPL with dCyd (pH 7.0-7.5, 37°C) gave 3-(carboxyethyl)deoxycytidine (3-CEdCyd) (9% yield) and 3,N^4-bis(2-carboxyethyl)deoxycytidine (3,N^4-BCEdCyd) (0.6% yield). 3-CEdCyd and 3,N^4-BCEdCyd were hydrolyzed (1.5N HCl, 100°C, 2 h) to 3-CEC and 3,N^4-bis(2-carboxyethyl)cytosine (3,N^4-BCEC) respectively. The structure of 3-CEC was assigned on the basis of UV and NMR spectra and the electron impact (EI) mass spectra of 3-CEC, and a tri-trimethylsilyl (TMS) derivative of 3-CEC as well as a deuterated (d_{27}) tri-TMS derivative of 3-CEC. The structure of 3,N^4-BCEC was assigned on the basis of UV spectra and the EI mass spectra of a tri-TMS derivative. Electron impact and isobutane chemical ionization mass spectra of 3-methylcytosine (3-MeCyt) and a di-TMS derivative of 3-MeCyt were obtained and were helpful in deducing the structures of 3-CEC and 3,N^4-BCEC. This is the first report of the alkylation by BPL of an exocyclic atom on a base in DNA. Compound 3,N^4-BCEC was not detected in BPL-reacted calf thymus DNA. The relative amounts of 1-(2-carboxyethyl)adenine, 7-(2-carboxyethyl)guanine, 3-(2-carboxyethyl)thymine and 3-CEC isolated from BPL-reacted DNA following perchloric acid hydrolysis were reported, and the major adduct isolated was 7-(2-carboxyethyl)guanine.

Goldschmidt has continued his investigation of potential carcinogenic N-nitroso derivatives of drugs. The N-nitroso derivatives of the drug propranolol (used in the treatment of hypertension and angina pectoris) and the drug hydrochlorothiazide (used as a diuretic and anti-hypertensive

agent) were prepared, and elemental analyses, chromatographic behavior and spectroscopic characteristics confirmed the structures of these two N-nitroso compounds. The drug phenylephrine (widely used as a nasal decongestant) was nitrosated, and a complex mixture of products was formed. One product isolated was the N-nitrosobenzoquinone derivative of phenylephrine.

The N-nitroso derivatives of the drugs, the parent drugs and some related substances are currently being tested for mutagenic activity in strains of *E. coli* and *S. typhimurium* both with and without rat liver enzymes. In other studies, the chemical structures of some penicillin G derivatives used to determine penicillin sensitivity in humans were established. A study of the hydrolysis and degradation of these penicillin G derivatives has also been started.

Dulak, N., Sellakumar, Snyder and Albert (Laboratory of Inhalation Toxicology and Carcinogenesis) have continued studies on the carcinogenicity of inhaled, direct-acting alkylating and acylating agents. Previous work has suggested that there is a direct, positive relationship between the chemical reactivity of direct-acting chemical agents and their carcinogenic potency in the respiratory tract of the Sprague Dawley rat. To further test the generality of this relationship, *in vitro* and *in vivo* studies have been initiated with six compounds.

Groups of rats are being exposed by inhalation to bis(chloromethyl)-ether, β-propiolactone, ethyl chloroformate, dichloroacetyl chloride, propylene oxide, or methyl methanesulfonate. These compounds were selected because they display a wide range of chemical reactivities. In companion studies, the same compounds are being examined in the C3H10T½ transformation assay system and in the V79 mutagenesis test system. The object of the *in vitro* tests is to correlate *in vitro* data, where complex physiological factors found in the intact animal are absent, with the *in vivo* results.

Sellakumar, Albert and Snyder (Laboratory of Inhalation Toxicology and Carcinogenesis) have shown that the exposure of rats by inhalation to formaldehyde (14.6 ppm) and hydrogen chloride (10.7 ppm) produces nasal tumors. Since both chemicals are extensively used, it is important to learn whether the HCl, the formaldehyde, or the combination was responsible for the tumors observed. A detailed study is in progress in which groups of Sprague Dawley rats are being exposed by inhalation to formaldehyde (15 ppm) and hydrogen chloride (10 ppm), combined in the exposure chamber or pre-mixed before delivery to the chamber, formaldehyde or hydrogen chloride alone, or to air. This study is designed to examine the carcinogenic effects of these combined exposures, possible carcinogenic effects of the chemicals given separately, and possible promoting or co-carcinogenic effects by hydrogen chloride on formaldehyde.

Efforts are also being made by Solomon (Laboratory of Organic Chemistry and Carcinogenesis) to determine whether alkylating agents such as bis(chloromethyl)ether, which are produced by the reaction of

hydrogen chloride and formaldehyde, may also be present in the exposure chambers. It is possible that such alkylating agents may have been responsible for the tumors observed. However, a recent study sponsored by the Chemical Industry Institute of Toxicology has shown that formaldehyde alone at 15 ppm produces nasal tumors in rats exposed by inhalation.

Another study by Belman (Laboratory of Biochemical Toxicology) seeks to determine whether DNA damage, as determined by the S-1 nuclease assay, results when several different types of cells are exposed to either atmospheres of hydrogen chloride + formaldehyde, BCME, or formaldehyde alone. This assay requires that target cells be cultured. One of the proposed studies call for the exposure of rats to atmospheres to BCME, hydrogen chloride + formaldehyde, and formaldehyde alone, and the culturing of rat nasal epithelial cells for the S-1 nuclease assay. Efforts to date have focussed on the isolation and culture of rat nasal epithelial cells. The results have shown that cells with beating cilia can be cultured for several days. In addition, media conditions have been refined to retard the growth of fibroblasts. It is expected that nasal epithelium can be cultured, free of fibroblasts, by a selective plating method.

Albert and Snyder (Laboratory of Inhalation Toxicology and Carcinogenesis) have focused attention on the effects of inhaled benzene on the pluripotent hematopoietic stem cell (CFUs) and the granulocyte-macrophage committed stem cell (CFUc). A dose-response study was conducted consisting of 6-hour exposures delivered over 5 consecutive days to male CD-1 mice. The lowest concentration which produced an effect on stem cells was 103 ppm. At this concentration, the numbers of femoral and splenic CFUs and splenic CFUc per organ were depressed. The numbers of CFUc/femur were depressed at 306 ppm.

In another study, the effects of 10 ppm benzene (the current TLV) delivered for 6 hours per day for 50 days were evaluated. Although the numbers of CFUs/femur were unaffected, the numbers of CUFs/spleen were elevated. A third study evaluated the effects of 300 ppm benzene delivered over 26 weeks. This exposure regime produced depressions in marrow and splenic CFUs and CUFc as well as a depression in splenic CFUc colony/cluster ratio. This finding is particularly significant in that it is suggestive of incipient myeloid leukemia.

The hematotoxic effects of inhaled benzene combined with ingested ethanol were also studied. C57BL mice were exposed to 300 ppm benzene for 13 weeks and also supplied with 0, 5, or 15% ethanol in their drinking water. Appropriate controls for all treatment groups were also maintained. It was found that the combination of inhaled benzene and ingested ethanol produced a greater hematoxic response than the inhaled benzene alone. Ingested ethanol alone, however, was found to have no effect on the monitored parameters. When compared to groups of mice receiving benzene alone, groups receiving benzene and ethanol showed depressions of peripheral lymphocytes and erythrocytes, femoral and splenic nucleated

cellularity, marrow normoblasts, marrow and splenic lymphocytes, and marrow granulocytes. In addition, there was an elevation of peripheral and splenic normoblasts in groups receiving benzene and ethanol. The appearance of normoblasts in peripheral blood is exceedingly rare and may be indicative of a severe hemolytic anemia.

Current work with benzene is directed toward the development of an animal model for benzene-induced leukemia and/or lymphoma and various inhalation exposure regimes using rats and mice are currently underway.

Sellakumar and Albert (Laboratory of Inhalation Toxicology and Carcinogenesis) have completed a study designed to evaluate the carcinogenic effects of the nickel compound 2'2-thiobis(4-t-octylphenyl-ato)n-propylamine nickel II (UV-1084), in cholesterol base, given by the intrabronchial pellet implantation technique.

Groups of 100 rats received pellet implants containing either UV-1084 in cholesterol base or cholesterol alone, and after one year, the mortalities for the two groups were 5 and 8%, respectively. The experiment was terminated after 115 weeks, and no carcinogenic effects were observed in any segment of the respiratory tracts. Non-malignant lesions were found in the respiratory tracts of animals in the two groups, but they were similar. The major pathological change found at the implantation site was bronchiectasis, which was frequently accompanied by inflammatory changes and fibrosis in the peribronchial areas.

Tumors were seen in other organs, but there were no differences between the treated and the control groups.

Rossman, Stone and Albert (Laboratory of Experimental Medicine) are continuing investigations into the mechanism of UV-mutagenesis in animal cells, in experiments designed to test whether UV-mutagenesis in animal cells requires an inducible error-prone DNA repair system similar to the "SOS system" in *E. coli*. If an inducible repair system is required, then inhibition of *de novo* protein synthesis at either the transcription or the translation level should lower the UV-induced mutation frequency. To investigate this possibility, Chinese hamster (V79) cells were exposed to either DRB (5,6-dichloro-1-β-D-ribofuranosylbenzimidazole, a transcription inhibitor), cycloheximide, or puromycin for various times (3-6 hours) following UV-irradiation (2-8 J/m^2). It was found that post-irradiation treatment with DRB resulted in a reproducible enhancement of UV-induced mutagenesis, but post-irradiation treatment with either cycloheximide or puromycin resulted in decreased UV-mutagenesis. Thus, the frequency of UV-mutagenesis does not appear to be dependent upon an inducible error-prone DNA repair pathway, since all three agents share the ability to inhibit *de novo* protein synthesis.

In order to understand the effects of these inhibitors on mutation frequency, DNA synthesis in the presence of these agents was examined. DRB stimulated DNA synthesis in both irradiated and unirradiated cells.

On the other hand, cycloheximide and puromycin caused an immediate inhibition of DNA synthesis in both irradiated and unirradiated cells. The enhancement of the UV-mutation frequency by DRB may be due to stimulation of DNA replication on a dimer-containing template, causing mutations by misreplication, rather than by misrepair. Cycloheximide and puromycin inhibit DNA synthesis and may, therefore, allow more time for constitutive error-free DNA repair to occur, assuming that cycloheximide and puromycin would not inhibit repair replication.

Therefore, it appears that UV-mutagenesis in Chinese hamster cells is a function of post-irradiation DNA synthesis and is independent of post-irradiation mRNA or protein synthesis.

Rossman, Mallon and Albert (Laboratory of Experimental Medicine) are studying the mutagenic and comutagenic effects of various environmental agents. Under moderate exposure conditions, where survival is high, it has been found that bisulfite alone is not mutagenic in either eukaryotic (Chinese hamster V79) or prokaryotic (*Escherichia coli*) cells. However, bisulfite does act as a comutagen with ultraviolet irradiation. Bisulfite approximately doubles the mutation frequency in UV-irradiated Chinese hamster V79 cells, and it causes a greater than eightfold increase in Trp^+ revertants in UV-irradiated *E. coli*. The comutagenic effect occurs whether cells are exposed to bisulfite during or immediately following UV-irradiation. Kinetic studies of the comutagenic effect of bisulfite in *E. coli* show that the effect decays in a biphasic manner, with a rapid component having an apparent half-life of 15 minutes and a slower component of the comutagenic effect persisting for up to 120 minutes after UV-irradiation.

Experiments with several strains of *E. coli* having varying DNA repair capacities indicate that excision repair is necessary for a comutagenic effect by bisulfite. *In vitro* studies have shown that bisulfite (at optimal *in vivo* comutagenic concentrations) inhibits the activity of *E. coli* DNA polymerase I. The effects of bisulfite on the exonuclease activities of polymerase I are being investigated.

Other agents were examined for enhancement of the mutagenic effect of UV-irradiation, and ascorbate was found to exhibit an effect similar to that of bisulfite. The mechanism of ascorbate comutagenicity is under investigation.

Wiesner, Rossman and Troll (Laboratory of Biochemical Toxicology) have continued studies on the effect of the liver carcinogen, l-ethionine, on the induction of "SOS functions" in the *tif-1* mutant of *E. coli*. Previously, it had been shown that very low concentrations of ethionine blocked the induction of lambda-prophage at 42° and caused filamentation in a *tif sfi* derivative at that temperature. In the more recent studies, the effect of ethionine on the expression or error-prone DNA repair by the *tif-1* mutant was investigated. Raising the temperature to 42° after a low dose of UV irradiation resulted in an increase in the induced

mutation yield, and this enhancement was blocked by 10 mM ethionine. Ethionine also blocked the enhancement of the spontaneous mutation rate which is observed in *tif* strains at 42°. Since ethionine is known to cause hypomethylation of DNA in a number of biological systems, the effects seen in the *tif-1* mutant may have been due to changes in the methylation of DNA.

Rossman, Molina, Albert (Laboratory of Experimental Medicine) and Troll (Laboratory of Biochemical Toxicology) are studying the mechanisms of metal mutagenesis and comutagenesis. A number of metals such as chromate (chromium VI), arsenic and nickel have been shown to be human carcinogens. Unlike organic carcinogens, most metals, with the exception of chromate, are not mutagenic in standard microbial assay systems. This lack of mutagenicity may reflect technical problems in the systems used to measure mutagenesis, since some of the metals are highly toxic. Alternatively, it may be that the carcinogenic metals act by a different mechanism.

To study this problem further, an investigation of the mechanism of chromate mutagenesis in *E. coli* was undertaken to determine the role, if any, of inducible error-prone DNA repair processes (SOS system). Assays were performed for Trp^+ revertants, using the fluctuation test. In this procedure, mutiple samples of bacteria undergo many rounds of cell division in the presence of a non-toxic level of the metal. If a mutation (to Trp^+) occurs, a pH indicator changes color in that sample. This method demonstrated that 10 μM chromate was mutagenic to all strains of *E. coli* tested, including those carrying the *recA* or *lexA* (SOS^- repair deficient) mutations. At higher concentrations, chromate caused the induction of λ prophage. These results suggest that although chromate is an inducer of the SOS system in *E. coli*, its mutagenicity is not dependent upon that system and probably occurs via direct misreplication of DNA.

In contrast to chromate, arsenite was not mutagenic in either *E. coli* or Chinese hamster cells. Recent epidemiological evidence shows that arsenite may act as a cocarcinogen rather than as a primary carcinogen. Previous work from this laboratory demonstrated that arsenite can interfere with the repair of UV-induced lesions in *E. coli*. However, at the highest tolerated dose (1 mM), arsenite appeared to primarily inhibit the mutagenic SOS repair pathway. Recently, it was found that exposure of *E. coli* to lower concentrations of arsenite caused an enhancement of UV-induced mutagenesis. This enhancement occurred only in excision-proficient strains of *E. coli*. The absence of mutation enhancement by arsenite in strains lacking, or defective in, excision repair indicates that arsenite may interfere with excision repair. The finding that a *polA* strain exhibited little or no arsenite comutagenesis suggests that arsenite does not inhibit the incision step of excision repair. The effects of arsenite on other steps of the excision repair pathway (i.e., exonuclease, polymerase or ligase activity) are being examined.

The absence of mutagenicity by arsenite, together with its activity as a comutagen, suggests that arsenite is a cocarcinogen rather than a primary carcinogen. Experiments now in progress are designed to determine whether arsenite comutagenicity also occurs in animal cells.

Belman, Garte and Troll (Laboratory of Biochemical Toxicology) are studying the biochemical events affected by promoters and promotion inhibitors. A key enzyme in these events is phospholipase A_2, which has been characterized in mouse epidermis. The enzyme is similar to those found in other tissues. The optimal conditions for the study of the enzyme were pH 7-9 with 12 mM calcium and 0.1% taurodeoxycholate. PMA had no effect on the *in vitro* assay system, implying that it causes an indirect alteration of the enzyme *in vivo*.

The activation of phospholipase A_2 by PMA *in vivo* leads to the release of arachidonic and other unsaturated fatty acids which are metabolized to prostaglandins. Malondialdehyde (MDA) is formed in these reactions and is being assayed. PMA produces an increase in epidermal MDA content from 25 pmol/ug DNA to 45 pmol/ug DNA. This maximal rise is obtained 27 hours after PMA treatment.

Mellitin, which shares many properties with PMA, is neither a promoter nor a modifier of promotion by PMA.

Garte, Belman and Troll (Laboratory of Biochemical Toxicology) have studied in detail the stimulation of RNA synthesis in cultured Balb C/3T3 fibroblasts by the tumor promoter phorbol myristate acetate (PMA). The specific rate of [^{3}H]-uridine incorporation into newly synthesized RNA was measured by determining both the specific activity of the labeled RNA and of the nucleotide precursor pools. The results indicate that the PMA effect is dependent on the growth stage of the cultures. In growing (log phase) cells, no increase in RNA synthesis was observed at any time after PMA treatment. However, when cells were treated in log phase, allowed to reach confluence, and then assayed for their RNA synthesis rate, a 60-100% (statistically significant) increase was found in PMA-treated cultures. When confluent cultures were treated, no effect was seen unless fresh serum was simultaneously present.

Results from studies of uridine incorporation kinetics confirm that the observed increase in RNA specific activity is due to RNA synthesis, as opposed to increased uridine transport or metabolism. Data on PMA dose response and reversibility, serum requirements, and effects on cell growth have also been obtained.

Lippmann (Laboratory for Aerosol and Inhalation Research) and Jaeger (Laboratory of Toxicology) have initiated an asbestos fiber inhalation study focussed on the influence of fiber length on the incidence of lung fibrosis and bronchial and mesothelial tumors. The study design calls for two groups of 81 Fischer rats to be exposed to crocidolite asbestos with fiber diameters between 0.5 and 0.8 μm. The mass

concentrations of asbestos will be the same in both chambers, but the fiber lengths will differ. In one case, the maximum fiber length will be approximately 5 μm, while in the other the minimum fiber length will be approximately 15 μm. Initial efforts have concentrated on the construction and test of elements of the fibrous aerosol generator, and on refinement of the computer system to be used for measuring the progression of changes in pulmonary mechanical function in the exposed animals. Work has also been initiated to modify four existing 1.6 m^3 exposure chambers in the inhalation laboratory to permit remote handling of the exposed animals in each of the two exposure chambers and their interconnected housing chambers.

Segal and Wortman (Laboratory of Organic Chemistry and Carcinogenesis) have been studying the nonhistone nuclear proteins in mouse epidermal nuclei. Previously reported attempts to isolate epidermal nuclei have yielded preparations contaminated by cytoplasm and other debris, and it was therefore necessary to first develop a procedure for the isolation of a pure nuclear preparation. This was accomplished by homogenizing epidermis in dilute citric acid and filtering the homogenate through a series of microporous nylon mesh filters. Final purification was acheived by banding the nuclei in a Metrizamide density gradient.

Albert, Burns (Laboratory of Experimental Medicine) and Altshuler (Biomathematics Laboratory) are continuing their studies of dose-response and dose-time relationships of tumorigenesis. These studies are being done in mouse and rat skin and in hamster lung. To date, the studies have found that in mouse skin, the binding of benzo(a)pyrene (B(a)P) with epidermal DNA and the initiation stage of carcinogenesis are consistent with a linear non-threshold dose-response relationship. The dose-response relationship for papillomas and carcinomas arising from the chronic application of B(a)P, however, shows an increasing slope, with tumor incidence being related to the second to third power of dose. A new quantitative theory of carcinogenesis is being formulated on the idea that tissues are inherently initated and promoted, and that carcinogens augment both aspects. Initiation is a linear function of dose, and promotion is a time-dependent process.

Other work in the laboratory involves evaluation of carcinogen interactions based on mouse skin initiation, the quantitative aspects of mouse skin initiation by transplacental exposure to DMBA, and the effects of *cis*-retinoic acid on the inhibition of mammary carcinogenesis.

Burns and Albert (Laboratory of Experimental Medicine) are comparing the interactions of diverse carcinogens, such as ionizing radiation, ultraviolet light and polycyclic aromatic hydrocarbons with epidermal DNA. Extensive *in vivo* data on the induction of skin tumors by different doses and treatment schedules of ionizing radiation have been fitted to a two-event model of carcinogenesis, where at least one event is repairable. Under the assumption that the initiating event in carcinogenesis is some form of damage to the DNA, the known rate of repair of oncogenic

damage was compared to the rate of repair of a candidate lesion, i.e., DNA single strand breaks. The single strand breaks were found to be repaired far too quickly for them to be identified as the major initiating event in the model. Double-strand breaks that interact to form chromosome abnormalities are now being considered as a possible candidate lesion.

One important issue in the multievent model of carcinogenesis is whether the required events might be produced by different chemical or physical carcinogens. In combined exposures of rat skin to ionizing radiation, ultraviolet light and a polycyclic aromatic hydrocarbon, the evidence so far supports the concept that events produced by different carcinogens do not interact.

In separate experiments designed to test temporal additivity, rat skin is being exposed to weekly doses of ionizing radiation. So far nearly a 10-fold increase in the tolerance of skin to the oncogenic effect of the radiation has been observed, compared to what would be expected if the same total dose were given in a single exposure. Modifiers of carcinogenicity, such as retinyl acetate, an inhibitor of tumor development and BUDr, a thymidine analogue sensitizer, have been utilized as probes of the relative importance of DNA damage and later development on radiation carcinogenesis.

Other experiments include the effect of age and radiation on the repair of DNA breaks, and the comparative response of mouse and rat skin to the cytotoxic and carcinogenic effects of ionizing radiation.

3. Research in Radiation Carcinogenesis and Dosimetry

Studies in progress in this area include the investigation of ways to improve radiation measurements, the use of model and *in vivo* systems to study the distribution patterns of radionuclides in organs and in bone, and the study of *in vivo* radionuclide metabolism.

Laurer, Karron and Cohen, N. (Laboratory for Environmental Studies) are conducting evaluation studies on a new type of photon detection system which can provide magnitude and positional data for a radioactive source without the use of a high-Z collimator. The detector is comprised of interleaved wafer sections of NaI(Tl) and CsI(Na), the former being recessed, so that only photons whose angle of incidence is normal to the detector or nearly so may excite the NaI(Tl) elements. When the common PM tube is appropriately masked, five delineable peaks are obtained with the detector centered over a source, because of the optical and scintillation characteristics of the instrument and the parameters of the amplifier. Multiple products of the background-corrected counts for the NaI(Tl) peaks are an extremely sensitive, symmetrical function of source position, while the promontory CsI(Na) wafers provide simultaneous quantitative activity information.

Extensive detector response data were obtained using a converted Nuclear Chicago Pho-Dot Scanner as an automatic positioning device for the detector, operating in conjunction with a specially developed computer-activated control unit. Under automatic program control, the detector covered a 144 cm^2 field about the point source in 5 mm steps, indicating on a grid the exact coordinates of each measurement location, and the computer provided an on-line data reduction printout for the spectra, including multiple products and propagation of the error data.

These data are presently being used to synthesize the performance of a 3x3, nine-detector array configured so that the orientation of the crystal wafers comprising each detector can be alternately rotated by 90 degrees. This represents one of the possible configurations that could optimize the scanning process.

Earlier studies were conducted on the behavior of NaI(Tl) wafers having different thicknesses. The results indicate that the 7.6 mm wafer dimension utilized in the detector is optimal in terms of resolution and other characteristics.

Cohen, N., Neton and Laurer (Laboratory for Environmental Studies) have recently completed construction of matched human skull phantoms for calibration of plutonium isotopes deposited in the head bones to provide an indirect means of estimating other skeletally-deposited plutonium. A plutonium-impregnated thoracic skeletal phantom has been designed which will simulate the distribution of plutonium in the baboon skeleton. This is the second set of skull/thorax calibration structures to be constructed. During this past year an americium (^{241}Am) skull/thorax phantom set was completed which has been successfully utilized to estimate the contribution of ^{241}Am in the thoracic skeleton to lung counts of

three exposed individuals. To more accurately estimate such contributions, experiments are now underway to determine a subject's skeletal mass through use of chest X-rays, ultrasonic scanning devices and various anthropometric measurements.

In addition, the whole body counting computer capability has been upgraded to expand capabilities for interactive modes of data processing. The major addition to this system is a telex communications to the CDC 6600 computer at the Courant Institute of Mathematical Sciences (CIMS). With this addition, it is possible to make use of the extensive existing software at CIMS. Now interlaboratory comparisons are facilitated for a rapid exchange of calibration and count-rate data.

Cohen, N., Wrenn and Lipsztein (Laboratory for Environmental Studies) have completed research designed to develop a model that can be used to calculate the accumulation of uranium in different organs of the human body for different kinds of exposure. The metabolic model derived divides the human body into several different compartments; *viz.* plasma, red cells, "short-term" bone, "long-term" bone, kidney, and urine. An exact analytical solution to the model has been proposed that can be solved for any kind of time-dependent exposure to uranium. For the experimental part of the study, five baboons were injected intravenously with uranium nitrate. The partition of uranium between plasma and red cells, its half-time in "short-term" bone and its distribution in soft tissues as a function of time, were each determined and utilized as model parameters for describing the uranium metabolism.

Harley and Pasternack (Laboratory of Biostatistics and Epidemiology) have continued work on the measurement of environmental levels of radon, and on bronchial and skin dosimetry of radon daughters. Also, a study was performed to measure the X-ray dose distribution to the skin of the head and neck in a typical tinea capitis irradiation. The X-ray dose study complements epidemiological work with tinea capitis patients that has now identified 82 basal cell carcinomas (BCC) in 2,227 persons. A further study was begun to investigate the possible x-ray dose received from cathode ray tubes while operating word processing devices.

A radon monitor has been developed and tested in the laboratory which is capable of measuring environmental levels of ^{222}Rn without interference from radon daughters. The detector operates with an electrostatic field (electret) that collects daughters as they form and removes them from the sensitive counting volume. Four field units are now under construction which will allow the start of long-term measurements of indoor and outdoor levels of radon.

Dosimetric modeling and the risk model for lung cancer induction by environmental levels of radon daughters were utilized to examine the proposed ICRP occupational limit for radon daughters. The new dosimetric model is based on bronchial morphometry developed by Yeh and Schum, who reported dimensions for the conducting airways in all 5 lobes of the male human lung. Based on these data, alpha-dose conversion factors in rads/working level month (WLM) have been calculated for each airway

generation in an atmosphere similar to that of an underground mine. This dosimetric approach, as well as the estimated lifetime risk of lung cancer for a 30-year exposure to the proposed ICRP limit of 5 WLM per year, indicate that the ICRP limit is not conservative.

Data reported by Kunz in Czechoslovakian uranium mines for deposition of radon daughters on skin have been used in a dosimetric model to estimate the skin dose from environmental levels of radon daughters. The calculations indicate that skin receives the highest adsorbed alpha dose of any body tissue. Attempts are now being made to verify the skin deposition fractions.

The results of X-ray studies using a child's head phantom indicate that the highest dose received was to the hair-covered scalp (400 rads). A preliminary dose-response relationship has been derived, based on normalized results (BCC per cm^2 per rad), and it appears that two expressions may apply. One expression applies to the hair-covered scalp, where there is no UV-radiation enhancement of carcinogenesis and the other applies to the face and neck, where there is possible UV-radiation enhancement.

Cohen, N. and LoSasso (Laboratory for Environmental Studies) have completed studies of the metabolism of $^{243,244}Cm$ citrate in six adult female baboons for periods of over two years after intravenous injection. *In vivo* measurements with gamma-ray scintillation detectors were used to estimate the parameters of deposition and retention of $^{243,244}Cm$ for the total body and for specific organ sites. In addition, $^{243,244}Cm$ was measured in urine, feces, blood, and biopsy samples of liver. Curium-243,244 leaves the blood rapidly, and 0.7% of the injected dose (ID) is present in the blood 24 hours after injection. The primary sites of early deposition are skeleton (≈ 60% ID) and liver (≈ 20% ID). The retention of $^{243,244}Cm$ in bone is protracted with half-time of several years, while that in liver is about 40 days. Approximately 7% of the injected dose is excreted in the urine during the first 24 hours. Curium-243,244, and ^{241}Am eliminated from the liver appear to be predominantly excreted in feces.

In addition to these studies of "normal" metabolism of curium, the effects of chelation therapy using the Ca or Zn salts of DTPA have been researched to include the determination of efficacy at different dose schedules and possible toxic effects due to trace metal depletion.

In experiments designed to define the distribution and retention of different actinide nuclides in the non-human primate, it was noted that, in units of concentration, the aorta may represent the most significant long-term, soft tissue deposition site in the body.

Five adult baboons injected intravenously with ^{241}Am and five injected with $^{243,244}Cm$ (both complexed as the citrate) were sacrificed sequentially at approximately 1 day, and 1, 3, 7, and 27 months thereafter. Throughout most of the 27-month period of exposure, concentrations of curium and americium were considerably greater in the aorta than in all other soft tissues including lymph nodes. At 27-month post injection,

the concentrations of curium and americium in the aorta were three and ten times, respectively, those in the liver.

Harley and Cohen, B. (Laboratory of Biostatistics and Epidemiology) are finishing a collaborative study with researchers at the University of Kentucky and at the U.S. Department of Agriculture. Female Fisher rats were exposed for a period of 6 months to smoke from cigarettes that were highly enriched in ^{210}Po. Control rats were exposed to standard cigarette smoke, and all rats were sacrificed at approximately 4-week intervals for 12 months. There was no exposure to cigarette smoke during the second 6-month period. The sacrificed animals were autopsied and the airways were examined by the track-etch technique to determine the spatial distribution of the alpha activity both on the tracheal mucosa and at the carina. The samples were exposed for periods ranging from 100 to 300 days, the detectors were etched to reveal the alpha particle tracks, and the track densities were determined. In addition, the parenchymal tissue from each animal was analyzed radiochemically for ^{210}Po and the rate of accumulation of the ^{210}Po during the 6-month cigarette exposure period and the subsequent rate of clearance were determined.

Cohen, B. and Harley (Laboratory of Biostatistics and Epidemiology) have measured the concentration of ^{210}Pb in both the tracheobronchial tree and the parenchyma in human autopsy specimens which had previously been analyzed for ^{210}Po. Measurements of ^{210}Po in the lungs of ex-cigarette smokers, who had not smoked for at least five years prior to death, suggested that there was long-term retention of inhaled ^{210}Pb. Samples were stored for approximately two years after the initial analyses of ^{210}Po to permit ingrowth of ^{210}Po from its ^{210}Pb grandparent, and the samples were then processed using ^{209}Po as a tracer for chemical yeild. Polonium-208 tracer had been used for the original ^{210}Po determinations. The polonium isotopes were resolved by alpha spectrometry, and thirteen lung specimens were analyzed, including samples from 5 smokers, 5 non-smokers, and 3 ex-smokers.

The ^{210}Pb concentration in the pulmonary parenchyma was found to be greater in smokers and ex-smokers than in nonsmokers. Therefore, a fraction of the ^{210}Po concentration previously measured in these same samples was supported by ^{210}Pb. Additional sources of ^{210}Po studied were the skeleton, diet, air, and cigarette smoke. The measurements permit apportioning of the excess ^{210}Po found in the lung parenchyma among the various sources. Good agreement was found between calculated values, using a hybrid lung model for the inhaled fraction, and the measured concentration.

4. Research in Respiratory Disease and Aerosol Physiology

Studies in progress are designed to determine the factors which affect aerosol deposition, translocation, and clearance from the respiratory tract. The work involves the use of models, both static and dynamic, and *in vivo* test systems.

Lippmann, Schlesinger and Gurman (Laboratory of Aerosol and Inhalation Research) have completed a series of studies of aerosol deposition in multiple replicate hollow human airway casts extending from the larynx to 3 mm diameter bronchi in which the intrabronchial patterns of aerosol deposition under steady and simulated inspiratory flows were compared. A preliminary analysis of the data for 3 μm and 8 μm MMAD particles indicates that deposition efficiency is dependent upon the Stokes number and other, still unidentified, factors.

Microscopic measurements of the surface density of deposition along the major bronchial bifurcation were made for various flow conditions. Some differences in deposition were found between constant and simulated inspiratory flows. For both cases, however, deposition was enhanced along the carinal edges of the airway bifurcations. The results can be used to construct more detailed and refined deposition models for toxicant and radionuclide dosimetry.

Airflow measurements are continuing in order to relate differences in overall intrabronchial deposition, as well as the microdistribution along the major bronchial bifurcation, to differences in local and overall airflow patterns. In addition, a study of particle deposition in casts under expiratory flow has been initiated in order to further improve the modelling of deposition within the tracheobronchial tree.

Lippmann, Rosenthal (Laboratory for Aerosol and Inhalation Research) and Palmes (Laboratory of Toxicology) are continuing to evaluate an aerosol probe technique for the *in vivo* measurement of airway dimensions. The technique derives airway sizes from the persistence of an aerosol in exhaled air after a period of breath holding. For verification and calibration, this method is being applied to freshly excised lungs of human accident victims. The lungs are first characterized by mechanical function tests, then tested with the aerosol probe, and finally sectioned and cast for measurement of alveolar airspace and bronchial airway sizes.

Two artificial thoraxes (one for human lungs and one for donkey lungs) have been constructed and equipped with instrumentation to carry out the pulmonary function tests and several human lungs have been analyzed. Current efforts are directed at determining the optimum test protocol for the measurements. To facilitate the airway size comparisons, a computer program has been written to calculate aerosol persistence as a function of particle size and airway dimensions, based on published anatomic models of the human lung.

Human subjects previously tested for lung deposition with a radioactive aerosol are also participating in *in vivo* tests with the aerosol probe. Since the conductive airway deposition in both aerosol tests depends upon airway sizes, the intercomparison will permit a further calibration of the aerosol probe technique.

Morris, Schlesinger and Lippmann (Laboratory for Aerosol and Inhalation Research) are continuing investigations on tracheobronchial mucus glycoprotein synthesis, secretion and transport in the rabbit, using fucose (6-deoxy-L-galactose) as a marker for these secretory glycoproteins. A sensitive analytical technique for fucose has been developed in which mucus is removed from the tracheobronchial airway lumen by bronchopulmonary lavage. Mucus glycoproteins removed by lavage and those remaining in the lungs following lavage will be purified by ultrafiltration and fractional ethanol precipitation. Quantification of the amount of mucous glycoprotein in these two samples and the incorporation of labelled precursors into the mucous glycoproteins of these samples will be used as measures of mucous glycoprotein synthesis and secretion. Net transport of mucus to the level of the larynx will be assessed by measuring these parameters in mucus collected at this level of the respiratory tract.

Lippmann and Robinson (Laboratory for Aerosol and Inhalation Research) are investigating the long term pulmonary clearance and translocation of magnetite ($FeO{\cdot}Fe_2O_3$), an inert aerosol, and coal dust, a cytotoxic aerosol, in rabbits. The dusts are neutron activated, and external measurements of thoracic particle retention are made periodically after the inhalation using both collimated scintillation detectors and new instrumentation for magnetic determination of pulmonary magnetite which was developed during the past year from mathematical models. The measured sensitivity of this new apparatus is 5 $\mu g/m^3$ ($FeO{\cdot}Fe_2O_3$) per picoTesla remanent field. With the available magnetometer (a fluxgate gradiometer) 0.5 $\mu g/m^3$ magnetite can be detected in the rabbit lung. Plans are underway to increase the sensitivity of detection to 25 ng/m^3 magnetite, with a more sensitive magnetometer being developed in collaboration with M. Acuña of the Goddard Space Flight Center, NASA.

Preliminary data obtained from a group of five rabbits have yielded the first magnetic observations of clearance during the tracheobronchial phase. A clearance $t_{\frac{1}{2}}$ of approximately 2.5 hours was measured for the first 24 hours after inhalation, and the $t_{\frac{1}{2}}$ for the alveolar phase (to one month) ranged from 9.5 to 12 days. Magnetic relaxation time, a function of the rotational freedom of magnetite within the lung, was very rapid following magnetization ($t_{\frac{1}{2}}$ approximately 2.5 minutes) but it declined to a $t_{\frac{1}{2}}$ of approximately 3 hours after 45 minutes. This suggests that some of the dust was still free on the alveolar surface, and that some had been bound (either by macrophages or in the interstitium).

The physical/mathematical model for magnetic clearance determination has been used to derive a technique of quantitative three-dimensional imaging of lung dust, and this method will be tested using both phantom lung models and rabbits.

In a coordinate study, Schlesinger, Leyko and Lippmann (Laboratory for Aerosol and Inhalation Research) are examining the effects of magnetite and coal dusts on the numbers and functions of the rabbits' alveolar macrophages. The animals are being exposed to low (10 mg/m^3) and high (500 mg/m^3) concentrations of these dusts, with a particle size of approximately 3.5 μm MMAD, for 1-2 hours.

The amount of dust retained, as a function of time, after inhalation exposure is being determined by the two non-invasive external measurement techniques cited above, i.e., the measurement of remanent magnetic fields within the thorax and the measurement of the γ-radiation from the neutron-activated particles using collimated scintillation detectors. Animals are sacrificed at specific time intervals and lung lavages are performed. The number of pulmonary alveolar macrophages (PAM's) present in the lung lavages, as well as the amounts of dust these cells have ingested, are determined. The retention measurements, along with the macrophage assay data, will be used to assess the contribution of macrophage activity to lung clearance.

Aliquots of PAM's from lung lavage are being maintained *in vitro*, and properties generally associated with healthy cultured macrophages are being examined. *In vitro* comparison of macrophage adhesion and mobility after prior exposure to cytotoxic and inert dusts provides information on the capacity of inhaled particles to alter macrophage participation in lung clearance.

5. Research in Epidemiology, Biostatistics, and Biomathematics

The Epidemiology program of the Laboratory of Biostatistics and Epidemiology seeks to relate the exposure of human populations to environmental factors to the incidence of disease and physical or performance impairment, with a view toward the identification and elimination of the causative factors. An Interlaboratory Epidemiology Unit has the function of providing guidance and recommendations for coordinating and integrating laboratory investigations with human population (epidemiologic) studies. This unit focusses the interdisciplinary talents of the Institute on collaborative research and the the stimulation of interaction between fundamental and practical problems in environmental health. The Laboratory of Biostatistics and Epidemiology is also concerned with the development of effective procedures for the design, analysis, and interpretation of epidemiologic experimental studies. The Laboratory of Biomathematics formulates mathematical models for exposure to environmental agents and for biological processes. Biomathematics also involves the extrapolation of animal data to humans for use in risk assessment.

Pasternack, Shore, Wales (Laboratory of Biostatistics and Epidemiology), Albert (Laboratory of Experimental Medicine) and Lippmann (Laboratory for Aerosol and Inhalation Research) are conducting an industry-wide study of about 2000 workers exposed to bis(chloromethyl)ether (BCME), to determine respiratory cancer mortality. The first survey of these workers conducted seven years ago showed a clear excess of lung cancer mortality among heavily exposed workers and a dose-response relationship. However, the risk at lower dose levels was unclear at that time. Because the data indicated an inverse relationship between exposure level and lung cancer latency, additional years of follow-up were necessary to characterize the risk at low exposure levels. Data are currently being assembled on the workers in the previous study to extend the follow-up an additional seven years, and an additional plant with a suspected BCME-associated excess has been added to the study.

Shore, Pasternack (Laboratory of Biostatistics and Epidemiology) and Albert (Laboratory of Experimental Medicine) have completed a mortality survey of about 1300 patients given arsenical therapy for syphilis in the early 1940's, to assess the relationship between cancer and arsenic at low doses. A similar group treated at about the same time with penicillin served as controls. Since the arsenic doses varied over a substantial range (0.03-3.0 g), the dose-response relationship could be examined for several cancer sites. Preliminary analyses have indicated a dose-related excess of genitourinary cancers. Although there were suggestive increases at other sites (respiratory cancer, brain, and multiple myeloma), they were not statistically significant.

Marmor, Pasternack, Shore (Laboratory of Biostatistics and Epidemiology), Lippmann (Laboratory for Aerosol and Inhalation Research) and Nelson have begun planning an historical cohort study to investigate the effects of cutting-oil exposures on cancer mortality in the bearing industry. The United Automobile Workers Union has provided access to records of workers employed from 1955 to the present in a large Connecticut

bearing plant. Cutting oils are used extensively in the manufacture of bearings and are of interest because natural cutting oils contain benzo-(a)pyrene, while synthetic cutting oils have been shown to contain nitrosamines.

Marmor, Pasternack (Laboratory of Biostatistics and Epidemiology) and Goldberg (Bureau of Child Health, New York City Department of Health) are completing data acquisition in a study of the effects of childhood lead poisoning on later neuropsychological performance. Forty-eight sets of twins who were discordant in childhood lead poisoning, according to records of the New York City Department of Health, have been tested and given physical examinations, and school and medical records are now being obtained. Analysis of the first 20 twin pairs indicated a significant deficit on the performance scale of the Wechsler Intelligence Scale of Children (Revised) among previously lead-poisoned children, compared with their less-exposed twin siblings. Data for the complete sample are currently being analyzed using both categorical techniques, treating the twins as historically lead poisoned or not lead poisoned, and regression techniques, using the historical blood lead levels of each twin as a quantitative measure of the degree of lead exposure experienced.

Evans, Daniel, Teal (Laboratory of Toxicology) and Marmor (Laboratory of Biostatistics and Epidemiology) are investigating long-term neuropsychological effects of childhood over-exposure to lead in twin siblings. Objective tests were first developed in studies with normal and intoxicated laboratory animals and then validated in studies with normal humans. The first eight pairs of twins studied revealed deficiencies in the learning of a complex visual discrimination that related significantly to childhood blood lead concentrations. The specificity of this intellectual deficit is being determined by comparing these results with results of tests of neuro-ophthalmological, motor, and clinical psychological functions.

Pasternack, Dubin (Laboratory of Biostatistics and Epidemiology), Strax (Guttman Breast Diagnostic Institute) and Hutter (St. Barnabas Medical Center, Livingston, New Jersey) are engaged in a study of the epidemiology of minimal breast cancer. One of the objectives of this study is to investigate by means of risk-factor analysis whether minimal breast cancer, in initially asymptomatic women, is essentially different from clinical breast cancer or whether it is an early manifestation of the same disease. A second objective is to compare prevalent to incident breast cancer cases, in order to identify risk factors associated with tumor aggressiveness. Data for this study are being provided by the Guttman Breast Diagnostic Institute, which offers free breast cancer screening to women living in New York City. The study group will consist of approximatley 1,000 cases and a random selection of approximately 2,700 controls.

Pathology materials and hospital reports have been requested from hospitals for all cases. Two independent histopathological reviews are being performed, one at New York University Medical Center and the other at St. Barnabas Medical Center. The results of the two reviews are being compared to determine whether or not there is agreement between

them. The evaluation is being made on the basis of key items which determine classification of disease as minimal noninvasive, minimal invasive (invasive component 10 mm or less), or clinical (invasive component greater than 10 mm). In addition, the histologic types of overall tissues and of the *in situ* and invasive components are being compared.

Computer programs are being used to detect obviously miscoded information, keypunch errors, and inconsistencies in all the computerized files, and to confirm the completeness of records for each participant in the study.

Pasternack, Shore, Koenig (Laboratory of Biostatistics and Epidemiology) and Strax (Guttman Breast Diagnostic Institute) are conducting a study of the relationship between hair dye use and breast cancer. In a previous study it was found that cumulative hair dye use showed a dose-response relationship with breast cancer, when the latent period was taken into account. However, the numbers were small, and a much larger study has been undertaken. The study group consists of approximately 500 women in whom breast cancer was detected, at the Guttman Breast Diagnostic Institute, and about 1000 randomly selected (control) women without breast cancer who attended the same screening center. The subjects are being interviewed to determine their history of hair dye use and their status with respect to the numerous risk factors for breast cancer. Latency effects and the potential confounding effects of other breast cancer risk factors will be taken into account in computing the relative risk of developing breast cancer among hair dye users. Dose-response relationships and interactions between hair dye exposure and other risk factors will also be assessed.

Pasternack, Dubin, Moseson (Laboratory of Biostatistics and Epidemiology) and Kopf (Department of Dermatology) are performing a case-control study designed to determine the degree of association between the occurrence of malignant melanoma and possible risk factors, including some which have not received quantitative analysis previously. The total data base will consist of approximately 1100 cases and a similar number of controls. Cases are being drawn from the tumor registry of the New York University Skin and Cancer Unit, while controls are being selected randomly from NYU Skin and Cancer Unit patients without melanoma or other neoplastic conditions. It is hoped that this study will lead to the identification of high risk groups that could benefit from future monitoring for malignant melanoma. Significant positive associations found in the study, which, if corroborated by others, may also be used to identify behavior patterns, which, if modified, could result in a decreased incidence of the disease.

Shore, Pasternack, Harley (Laboratory of Biostatistics and Epidemiology) and Albert (Laboratory of Experimental Medicine) have been following a group of 2200 persons given X-ray therapy for ringworm of the scalp (tinea capitis) between 1940 and 1959. An excess of both brain and thyroid tumors has been observed in the irradiated group, as compared to a control group of unirradiated tinea capitis patients. The excess

thyroid tumors occurred in response to a dose of only about 6 rads. Beginning 20 years post-irradiation, a large excess of basal cell skin cancers has been found on the head and neck. A dosimetry of the skin has been completed, and the results suggest that X-irradiation, ultraviolet exposure, and host susceptibility factors are all important components of the skin cancer excess.

Shore, Pasternack (Laboratory of Biostatistics and Epidemiology), Albert (Laboratory of Experimental Medicine), Hempelmann and Woodard (Department of Radiology, University of Rochester) have conducted a survey of about 600 women given X-ray therapy for acute postpartum mastitis between 1940 and 1955, and control groups consisting of siblings and unirradiated mastitis patients. A two-fold risk of breast cancer was found, beginning about 10-15 years after irradiation and extending through the maximum length of follow-up (35 years). The dose-response relationship was approximately linear up to a high dose level. Radiation did not appear to be synergistic with other risk factors for breast cancer. Recent joint analyses of these data and two other major studies of radiation-induced breast cancer have examined factors of: comparability of risk, age at exposure, tumor latency vs. dose, dose-fractionation effects, and shape of the dose-response curves. A third survey of these populations is nearly completed.

Shore, Pasternack (Laboratory of Biostatistics and Epidemiology), Hempelmann and Woodard (Department of Radiology, University of Rochester) have followed about 2,700 persons irradiated in infancy for thymic enlargement, with siblings as controls. Thyroid doses ranged from 5 to over 1000 rads, given in 1 to 10 fractions. Recent analyses for thyroid cancers showed both linear and quadratic (dose-squared) components to the dose-response curve, and there was a sparing effect of dose-fractionation. For thyroid adenomas, there was no evidence of a dose-squared component to the dose-response curve, and there was no sparing effect of dose-fractionation. Latency and host susceptibility factors for radiation-induced thyroid carcinoma were also examined. Another survey of these populations, providing an additional seven years of follow-up, has been completed and will soon be ready for analysis.

Marmor and Pasternack (Laboratory of Biostatistics and Epidemiology) have completed a study of Bergen County, New Jersey cancer mortality in cooperation with the Bergen County Department of Health and Environmental Protection. Age-standardized mortality rates and rates of change from 1962 through 1975 were calculated and compared with U.S. rates and rates of change for 10 major cancer sites. Male and female rates for respiratory cancer and the male rate for all cancer sites combined increased significantly more quickly in the United States than in Bergen County during the study period. Differences between Bergen County and the United States for these sites originally shown in the National Cancer Institute *Atlas of Cancer Mortality for U.S. Counties, 1950-1969*, have thus diminished until, in 1974-75, the difference between the two populations was negligible. The meaning of these results for local cancer education and prevention programs has been discussed both in a publication in press and with responsible officials in Bergen County.

geographical region or even an individual dwelling is not known. The only long-term radon daugher data reported so far are those of the U.S. Department of Energy, Environmental Measurements Laboratory (EML). They have reported three years of continuous outdoor measurements in Chester, New Jersey, obtained using the two filter method. Large seasonal variations in ^{222}Rn concentration, and diurnal variations are obvious in their measurements.

This laboratory has developed a portable continuous ^{222}Rn monitor which measures ^{222}Rn without interference from its alpha emitting daughters. The present design is based upon the doctoral dissertation of Passaporn Chittaporn of the Thai Atomic Energy Commission, a graduate of this department. The detector consists of a zinc sulfide-lined cylinder which is placed over a 12.7 cm photomultiplier tube. A permanently charged Teflon polymer (negative electret) is placed over the alpha phosphor lined cylinder, so that as positively charged ^{218}Po is formed from ^{222}Rn decay, it is collected by the electrostatic field and removed from the sensitive counting volume of the chamber. In this way, only the alpha particles emitted by ^{222}Rn contribute to the observed count rate in the chamber. The detector is housed in a light-tight outer housing, and ^{222}Rn diffuses passively into the system. The efficiency for counting ^{222}Rn is high, 170 counts per hour per pCi/l, and the inherent background of the system is low, 0.6 counts per hour. At an environmental concentration of 100 pCi $^{222}Rn/m^3$, the Poisson counting error for a one hour count is ± 30%.

Presently, four of these continuous monitors are being built to measure environmental levels of ^{222}Rn. Each portable monitor is self contained in a 30 x 30 x 76 cm outer housing and contains the detector and a miniature scaler-printer. The scaler-printer is adapted from a design developed at EML. Continuous long-term measurements will begin by the end of 1980, and indoor and outdoor levels of ^{222}Rn will be measured simultaneously and continuously at two locations for one year. Basic meteorological data will be collected such as temperature, pressure, humidity, and rain or snowfall. If possible, lapse rate will be measured along with the ^{226}Ra content of the soil in these locations. With this information, it may be possible to formulate a predictive method to obtain average annual exposure based upon short term measurements. Furthermore, with these data it will be possible to begin estimates of lung cancer related to environmental levels of radon daughters.

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Staff: S. Altman, B.A.

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7. Bronchial Dose from Radon Daughters in a Five-Lobed Human Lung

The common factor which relates radon daughter exposure in various environments and lung cancer risk is the alpha dose delivered to basal cells in bronchial epithelium. Alpha dose modeling in the bronchial tree depends primarily upon ten factors involving the physical characteristics of the atmosphere and biological parameters of the human lung. One important consideration is bronchial morphometry. All bronchial dose models, so far, have used simple dichotomous branching for the airway structure. Yeh and Schum have reported airway length and diameter for the entire five-lobed lung from two male subjects. These data allow more realistic modeling of the alpha dose to the bronchial airways.

Recent studies in this laboratory have utilized their morphometric measurements in dose modeling to obtain dose conversion factors (rad/WLM) for each airway. In this model, the highest alpha dose is still delivered to generation 4 (segmental bronchi). At this branching level, the left lobes receive an alpha dose approximately 20% higher than the corresponding airways in the right lobes.

ICRP is proposing a new annual limit of exposure of 5 WLM per year for occupational exposure to radon daughters. This proposed value was examined by two approaches. One approach was to look at the ICRP recommendations that annual mortality not exceed 10^{-4}, which is equivalent to mortality in other safe industries, and to compare this with a model of lung cancer risk from radon daughters. The other approach utilized the dose conversion factors for the five-lobed lung and compared the alpha dose with the ICRP effective dose equivalent of 5 rem per year. Both methods indicate that the proposed ICRP annual limit of exposure is too high by a factor of about two.

The dose conversion factors developed so far for the five-lobed lung apply to radon daughter exposure in underground mines. Current studies involve the development of factors which apply to environmental levels of radon daughters.

Sponsor: Institute of Environmental Medicine

Principal Investigator: N.H. Harley, Ph.D. and B.S. Pasternack, Ph.D.

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8. Metabolic Studies of Curium in the Adult Baboon: Retention, Distribution, Kinetics, and Enhanced Excretion by Chelation Therapy

The distribution and retention of curium (as $^{243,244}Cm$ citrate) has been measured in nine adult female baboons for periods ranging up to 26 months after intravenous injection. Dual-crystal NaI(Tl)-CsI(Tl) scintillation detectors were used to estimate values for the initial deposition and retention of curium in the total body, as well as at specific tissue and organ sites. The measurements were supplemented with the determination of curium levels as a function of time in urine, feces, blood, and in biopsy samples of liver and bone.

Curium-243,244 leave the blood rapidly, and only 0.7% of the injected dose (ID) is present in the total blood at 24 hours post injection. The mathematical expression describing the retention of curium in the plasma can be represented by $R = 0.45\ t^{-0.93}$ ($p = 0.98$), where R is the amount of $^{243,244}Cm$ in the plasma, t is the time after injection in days and p is the correlation coefficient.

The primary and most significant tissue sites of early deposition were the total skeleton (~20% ID at 24 hours) and liver (~20% ID at 24 hours). The retention of $^{243,244}Cm$ in the skeleton was long with a biological half-time of several years, while the $T_{\frac{1}{2}bio}$ in the liver was determined to be approximately 40 days. There is preliminary evidence from analyses of autopsy specimens than many soft tissues, including muscle, retain $^{243,244}Cm$ with half-times of several months, suggesting that translocation of $^{243,244}Cm$ from soft tissue to bone may have masked any early skeletal elimination of $^{243,244}Cm$. A fraction of the $^{243,244}Cm$ recirculated from soft tissue will be absorbed by bone, making it appear that the skeletal burden does not change during the first few months. Approximatley 7% of the injected dose is excreted via the urinary route during the initial 24 hours.

If it is assumed that $^{243,244}Cm$ appearing in feces, like ^{241}Am, results predominantly from activity eliminated by the liver, and that the entire liver burden is eventually excreted in the feces via a biliary pathway, the fecal $^{243,244}Cm$ excretion rate is directly proportional to the rate of loss from the liver. Clearance constants for $^{243,244}Cm$ in the feces of four baboons were consistent with values determined by external measurements of liver and whole body. Using the derived fecal excretion curves, with corrections made for early variations due to liver uptake, the cumulative fecal excretion of $^{243,244}Cm$ at infinity varied between 14 and 23% of the injected dose for these animals.

A comparison of actinide retention in the livers of rats, dogs, and baboons for ^{241}Am and ^{242}Cm or $^{243,244}Cm$ indicates a wide range of interspecies variability for each element, in terms of fractions deposited and retention times. In the rat, the accumulations of ^{242}Cm in liver and skeleton are 60 and 24%, respectively, of the injected dose when given intravenously as either citrate or chloride. In beagles injected intravenously with curium citrate, the liver and the skeletal burdens one week after injection were 35 and 41% of the injected dose, respectively. The metabolic characteristics of curium in the baboon are different from those of the rat or the dog. The skeleton: liver ratio of curium deposition at early times after injection is highest in the baboon, with only 20% in the liver and approximately 60% in the skeleton. Retention in baboon liver is more like that in the rat than in the beagle; the retention time of curium in the liver of the dog is long, on the order of years.

In addition to these studies of "normal" metabolism of curium, the effects of chelation therapy using the Ca or Zn salts of DTPA have been

studied to determine efficacy at different dose schedules and possible toxic effects due to trace metal depletion.

In experiments designed to define the distribution and retention of different actinide nuclides in the non-human primate, it was noted that, in units of concentration, the aorta may represent the most significant long-term, soft tissue deposition site in the body. Five adult baboons injected intravenously with ^{241}Am and five injected with $^{243,244}Cm$ (both complexed as the citrate) were sacrificed sequentially at approximately 1 day, and 1, 3, 7, and 27 months, thereafter. Throughout most of the 27-month period of exposure, concentrations of curium and americium were considerably greater in the aorta than in all other soft tissues, including the lymph nodes. At 27 months post injection, the concentrations of curium and americium in the aorta were three and ten times, respectively, those in the liver.

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9. Investigation of Polonium-210 as a Risk Factor in Smoking Related Carcinogenesis: Animal Studies

The ultimate distribution of the ^{210}Po inhaled in cigarette smoke results from a combination of deposition and clearance mechanisms. Measurement of the microdistribution of this alpha emitter on the mucosal surface of the conducting airways in human autopsy specimens revealed areas of elevated alpha activity (Cohen *et al.*, *Health Physics 39*:619, 1980) where the surface concentration was typically < 1.0 fCi/cm^2.

Animal experiments are now in progress to supplement the human studies. Fischer female rats were exposed at the University of Kentucky to cigarette smoke with highly elevated ^{210}Po content. Tobacco for the experimental cigarettes was grown by the USDA in a solution highly enriched in ^{210}Pb, and cigarettes were prepared after sufficient time had elapsed for ingrowth of the ^{210}Po granddaughter.

Animals were sacrificed and autopsied both during and after a 6-month smoke exposure period. Thus, the time course for the appearance of ^{210}Po activity on the mucosal surface can be determined. Freeze-dried specimens of trachea, carina, and nasopharnyx were exposed to track-etch film detectors for 100-300 days. The films are etched to reveal the alpha particle tracks, and scoring is underway. Samples taken from animals sacrificed during the smoke exposure period reveal high levels of activity on the tracheal mucosa and in the larynx, compared to controls.

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Principal Investigator: N.H. Harley, Ph.D.

Co-Investigator: B.S. Cohen, Ph.D.

10. Investigation of Lead-210, Polonium-210, and Plutonium-239 in the Lungs of Smokers, Non-Smokers and Ex-Smokers

Lead-210 in cigarette smoke provides a unique tracer for particle clearance, since it seems to have both a short-lived and a long-lived component in the parenchyma. Plutonium-239,240, present in all persons from global fallout is of interest since it is insoluble and is in the lung solely from inhalation, without the complication of systemic redistribution.

Thirteen whole human lungs were obtained at autopsy from 1976 to 1978. The lungs were frozen while expanded and vacuum dried in a specially designed freeze-drying unit (Virtis Company). The tracheobronchial tree was then dissected from the whole lung to about the sixth

branching level. The pulmonary parenchyma and one-half of the dissected tree were analyzed radiochemically for ^{210}Po. Polonium-208, lead carrier, and ^{242}Pu tracer were added to the samples prior to wet digestion in HNO_3 and H_2SO_4. Lead sulfide was precipitated using thioacetamide to separate ^{210}Pb and ^{210}Po from the sample matrix. The lead sulfide was dissolved in HCl, and ^{210}Po and ^{208}Po tracer spontaneously deposited on a 1.75 cm polished nickel disc for alpha spectrometric measurement. The ^{210}Pb, ^{210}Po was stored for two years, ^{209}Po tracer was added, and ^{210}Po was replated to determine ^{210}Pb.

The supernatant solution from the lead sulfide precipitate was reserved for solvent extraction analysis for $^{239,240}Pu$. The $^{239,240}Pu$ analyses were done by Dr. Isabel Fisenne of the Environmental Measurements Laboratory (EML).

Chemical yield for the ^{210}Pb was determined from the measurement of stable lead in the final sample solution. The lead content was measured by direct current plasma emission spectrometry by Robert Morse of EML. The stable lead yield combined with the plating yield, as measured by the ^{209}Po tracer, result in the overall yield for the Pb determination. For the pulmonary parenchyma, the average yield was 46 ± 11% for ^{210}Po, 30 ± 6% for ^{210}Pb, and 65 ± 10% for $^{239,240}Pu$. For the tracheobronchial tree, the radiochemical yields were 58 ± 8% for ^{210}Po, 37 ± 7% for ^{210}Pb and 60 ± 10% for $^{239,240}Pu$.

It is assumed that the concentration data are lognormally distributed and, therefore, best described by the median. For the parenchyma, median concentrations for smokers, non-smokers, and ex-smokers are 7.5, 2.8, and 6.8 fCi/g of ^{210}Po and 3.4, 1.6, and 10.0 fCi/g for ^{210}Pb, respectively. An overall median for all 3 groups of 0.07 fCi/g is observed for $^{239,240}Pu$.

Previous analyses have shown that concentrations of ^{210}Po in the tracheobronchial tree are not significantly different for the smokers, non-smokers, and ex-smokers. The overall median concentration is 3.2 fCi/g ^{210}Po. When the measurements for the tracheobronchial tree in smokers, non-smokers, and ex-smokers are combined, the median concentrations are 3.7 fCi/g for ^{210}Pb and 0.09 fCi/g for $^{239,240}Pu$.

The parenchymal ^{210}Po concentration is greater in smokers and in ex-smokers than in non-smokers. This is also true for the ^{210}Pb concentration. The ^{210}Po concentration is greater than the ^{210}Pb concentration in both smokers and non-smokers. Thus, ^{210}Po that is not supported by its grandparent, ^{210}Pb, is present in the parenchyma.

The excess ^{210}Po in the pulmonary parenchyma may be attributed to various sources. It is well known that the skeleton is a reservoir for ^{210}Pb in the body. Polonium-210 may also appear in the parenchyma following inhalation of this natural radionuclide from the air, and by gastrointestinal uptake from the diet. It may be possible to identify

these sources through the excess ^{210}Po concentrations measured in non-smokers and ex-smokers.

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Principal Investigators: N.H. Harley, Ph.D. and B.S. Cohen, Ph.D.

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D. RESPIRATORY DISEASE AND AEROSOL PHYSIOLOGY

1. Regional Particle Deposition in the Human Respiratory Tract

Although most studies of particle deposition in the lung are related to the total amount of material deposited, the health risk from many inhaled aerosols may be more related to the specific pattern of regional airway deposition. Since the pneumoconioses result from inhaled dusts which deposit in the alveolar region, most definitions of "respirable" dust have only considered those particles which penetrate through the conductive airways. However, several important chronic lung diseases relate to alteration in the bronchial airways due to deposition of irritant aerosols on the bronchial surfaces. Two notable examples are chronic bronchitis and carcinoma. Thus, for a complete understanding of potential risks from inhaled particles, it is essential to obtain information on the intrabronchial sites of enhanced deposition and the efficiency of deposition at these sites.

Many experimental systems have been used for the characterization of intrabronchial particle deposition patterns. In this laboratory, casts of the human tracheobronchial tree are used in conjunction with a laryngeal model through which monodisperse γ-tagged Fe_2O_3 aerosols are inhaled.

A series of tests has been completed which was designed to characterize deposition efficiencies and to locate deposition hot spots within the first few bronchial airway generations, for both constant and cyclic inspiratory flow regions. The study was performed using a destructive analytical technique which necessitated the production of a number of replicate hollow casts from a single solid master cast, in conjunction with a variable orifice larynx. The "destructive" analytical technique being used is capable of greater resolution and sensitivity than the previously used non-destructive technique which involves external measurements of airway segments with a collimated scintillation detector. Following aerosol exposure, the hollow airway cast is sectioned into premarked regions, and the retention in each segment is measured in a well-counter. The studies performed to date show that there are some significant differences between the aerosol deposition patterns in constant and simulated inspiratory flow.

One of the major differences can be noted in total tracheal deposition. Deposition in the trachea is strongly dependent upon the flow pattern through the larynx and appears to be determined by particle inertia, with some contribution due to turbulent diffusion. The wedge shape of the larynx contributes to this influence by forcing a considerable fraction of the inspired air to the rear of the trachea. Tracheal deposition is greater under constant flow than under simulated (cyclic) inspiratory flow. This may be related to the change in Stokes number (i.e., the inertial parameter) with changes in laryngeal size during the inspiration cycle. As the larynx opens during the simulated inspiratory

cycle, less of the total air entering is forced to the rear of the trachea, and the Stokes number is slightly reduced. Tests performed at identical flow rates under constant flow conditions with changes in laryngeal size are in agreement with these conclusions. Therefore, with larger flows, a smaller proportion of the aerosol was observed to be deposited in the trachea. These observations are consistent with previous measurements of tracheal deposition efficiencies in the cast under the cyclic inspiratory flow. Previous *in vivo* tracheal deposition data show comparable tracheal deposition efficiencies.

In analyzing the deposition data beyond the trachea under identical flow rates at constant flow conditions with altered laryngeal size, there were few, if any, differences in overall deposition efficiencies in the subsequent generation. Because of these observations, the tracheal generation has been segmented from the other generations. Analysis of factors affecting deposition was initially confined to the 1st to 5th generations. Although a significant correlation can be obtained by incorporating the tracheal Stokes number alone ($r = 0.87$), it appears, upon closer investigation, that a stronger correlation can be observed in each of the individual generations, indicating that some morphometric parameter must be considered to further modify the Stokes term. This observation is consistent with the present study as well as previous studies performed in this laboratory by Schlesinger and Chan.

Differences have been observed under all flow conditions with the total generational deposition efficiencies being somewhat greater under cyclic inspiratory flow conditions than under constant flows. The differences observed were directly related to flow and inversely related to particle size, since the dominant deposition mechanisms for the particles used in this study are inertial impaction and sedimentation. If the flow profiles are the same, deposition by impaction should be the same under both constant flow and the cyclic regime, having equivalent mean flow. This may be accounted for by the relatively small relaxation times of the 3 and 8 μm particles, compared with the time for a single inspiration. Thus, inertial impaction may be considered to be a quasi-steady process, and efficiency of deposition obtained with constant flow should be equivalent to that obtained in cyclic flow tests run at an equivalent mean flow.

Particles of the size used in this study may also deposit by sedimentation. Deposition by this mechanism is proportional to the residence time of the particles in the airways. Residence time, in turn, is proportional to flow rate. Since the mean flow of the cyclic regimen equals the constant flow, the mean residence time for a specific size particle under the two regimens would be identical. Therefore, if the flow profiles are the same, the probability of deposition by sedimentation from a constant flow and a cyclic flow at an equivalent mean flow rate should be equivalent.

If deposition efficiencies due to both impaction and sedimentation should be equal for the constant and paired cyclic tests, then the observed differences in deposition for both particle sizes between the two flow regimens must be due to some factor affecting deposition which differs between constant and cyclic flow, such as the degree of turbulence or localized velocity profiles.

Since flow through the laryngeal-tracheobronchial cast system clearly has a significant effect on aerosol deposition, a study involving the measurement of velocity profiles and turbulence intensities in the larger airways of the hollow cast was undertaken. The system consists of two miniature hot-wire anemometer probes incorporating a special positioning mechanism. Airflow measurements under constant and simulated inspiratory flow through the hollow tracheobronchial tree cast, connected to the variable-opening larynx, are expected to provide information which will permit an analysis of the factors influencing intrabronchial deposition patterns. Preliminary evaluation has indicated that the division of total flow between the inner and outer halves of the main bronchi, and presumably, other branches in the cast, was the same for both constant and cyclic flow, with the bulk of the flow moving in the inner section. Thus, a greater proportion of aerosol should also exist in the inner section of the airways under both flow regimens. However, under cyclic flow, the turbulent intensities in this half of the airway are somewhat greater, especially near the inner wall. Secondary flows generated at the bifurcations act to concentrate the particles toward one side of the airway. This, coupled with the higher level of turbulence, should promote the passage of particles through the boundary layer, thus increasing observed deposition efficiency.

In order to evaluate any differences in the pattern of aerosol deposition at the bifurcations, selected major bronchial bifurcations were mounted and analyzed microscopically using a Bausch and Lomb Stereo-zoom microscope. There was very little difference in the microscopic deposition patterns. Deposition was, as expected, greatly enhanced along the carinal edges. Constant flow results in denser deposition along the carinal ridge, especially at the lower flow rate. In addition, greater particle density occurs towards the ventral wall of the airway under constant flow, while under variable flow, the tendency is for greater surface density toward the dorsal wall.

A study of aerosol deposition under expiratory flow has been initiated to obtain a more realisitic view of total aerosol deposition in the tracheobronchial tree during a full respiratory cycle. This study is being performed with two identical hollow tracheobronchial casts connected in series at their terminal branches.

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2. Use of Aerosols for the Measurement of Airspace Sizes

The relationship of airway size to pulmonary aerosol deposition is important in inhalation toxicology for two reasons. First, airway size affects deposition, which may in turn affect susceptibility to tissue damage caused by the deposition of toxic agents. Second, when monodisperse aerosols are inhaled according to standardized exposure protocols, measurement of their deposition may be used to infer the average sizes of the airways in which they were suspended. The onset of lung disease can be characterized by changes in the mechanical functions of the lungs. Some of these changes can be directly related to changes in airway and airspace dimensions, as revealed by examination of diseased lungs at autopsy. However, these changes do not usually affect the results of functional testing until disease has substantially progressed, in many cases to an irreversible stage. A sensitive and more direct measurement of airway and airspace dimensions would be useful for the early detection of lung disease and could be used as a screening tool in environmental or occupational settings. In addition, it could be used to determine the normal range of variation in airway dimensions and thereby facilitate more precise estimation of the degree of deposition of toxic agents in a population.

An aerosol technique for characterizing average alveolar airspace sizes which was originated by Palmes *et al.* is undergoing further development and refinement for use in the characterization of both conductive airway and alveolar airspace sizes. Basically, this technique derives airway sizes from the persistence of an aerosol during a series of periods of breathholding. When the aerosol persistence is plotted vs. breathholding time, the initial slope of the curve can be related to alveolar deposition. The y intercept (0 breathholding time) corresponds to the initial deposition that takes place in the conductive airways, primarily those in the more distal parts of the tracheobronchial tree. The first slope gives a measure of average airspace size, primarily in the parenchyma. Variations in alveolar dimensions, as estimated by this technique, have been shown to be correlated to disease states. A second slope appears at long breathholding times. This is probably related to particle sedimentation within the larger airways. The y intercept and the second slope show promise as indicators of bronchial dimensions.

Direct calibration and verification of this technique is necessary, since the derivation of airway sizes from the aerosol persistence curves rests on several assumptions which are difficult, if not impossible, to verify experimentally. These include the nature of the settling process which occurs during breathholding, the distribution of the aerosol within the airways of the lungs on inhalation, and the relative penetration of the aerosol into the various airway generations.

In order to establish a closer relationship between airway dimensions measured with the aerosol probe and the actual geometric dimensions, a series of experiments has been started using excised human lungs. The lungs are first characterized by mechanical function tests, then tested with the aerosol probe and finally sectioned and cast for direct measurement of airway and airspace sizes. The lung function tests are used to identify any diseased or otherwise abnormal lungs and to correlate normal variations in these parameters with the results of the aerosol probe. The lungs being tested were removed from accident victims, and include adult smokers and non-smokers as well as children. This will permit direct estimate of the variation in airway sizes among normals as a function of smoking history, and between children and adults.

Measurements have already been made on five pairs of lungs. The subjects were all male, ranged in age from 17 to 34 years and included 3 smokers and 2 non-smokers. Mechanical lung function parameters were measured by placing the lungs in an artificial thorax. Negative pressure applied either with a vacuum pump or a large syringe was used to achieve inflation of the lungs. From simultaneous measurements of pressure, flow, and volume, it was possible to determine static and dynamic compliance, and pulmonary resistance. The methods are analagous to *in vivo* measurement of the same quantities. The lungs were also tested with a single breath nitrogen washout, a test which measures uneveness in the filling of the lungs and is used clinically as an indicator of small airway disease. As expected, the range of values for the parameters

of the excised lungs is somewhat different from that measured *in vivo*, but the results are reproducible within a given pair of lungs, and, as a group, fall within a consistent range.

Using the same artificial thorax, the lungs have also been tested with the aerosol probe, and characteristic curves of aerosol persistence vs. breathholding time were obtained and are being analyzed. To facilitate the analysis, a computer program has been written which sums the contributions to deposition of the various generations, assuming published anatomic models of the human lung. Three of the lungs have been preserved in a freeze drying apparatus to prepare them for thick sections from which morphometric measurements will be made.

A number of other measurements are also being undertaken to further evaluate the aerosol probe. Human subjects previously tested for lung deposition with a radioactive aerosol are being tested with the aerosol-breathing probe. Since both measurements depend upon airway sizes, this will permit a further calibration of the aerosol probe. Preparations have also begun for the comparison of donkey lungs measured *in vivo* with a radioactive aerosol and the same lungs, excised after sacrifice, evaluated with the breathholding probe and with morphometric measurements. An artificial thorax suitable for these lungs has been constructed and is being tested.

The human *in vivo* deposition measurements with breathholding have continued. In an effort to obtain information as to the average sizes of small airways, three approaches have been taken: 1) inhalation of aerosol to just fill the conducting airways, 2) longer periods of breathholding (to approximately 70 seconds, rather than 30 seconds), and 3) use of an aerosol with a larger median diameter than the 0.6 μm used in previous studies. The rationale for the three methods is the same, to eliminate the contribution of the pulmonary region aerosol deposition from the decay curve, so that the sedimentation of the aerosol in the small conductive airways will be more obvious.

The first approach was the least successful because of the variability between subjects and the difficulty in accurately determining anatomical dead space. Thus, the appropriate volume of aerosol to be inhaled could not be accurately determined. Neither of the other two methods alone was found to be satisfactory in providing information as to the average size of small airways. Rather, both time of breathholding and the median diameter of the aerosol were increased to about 70 seconds and 1.5 μm, respectively. Some modification of the TPP aerosol generation apparatus was necessary to produce the larger diameter particles.

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3. Mucociliary Particle Transport in the Tracheobronchial Airways

Insoluble particulate material is cleared from the conducting airways of the lower respiratory tract by the tracheobronchial mucociliary clearance system. Many agents, for example, cigarette smoke, sulfuric acid aerosol, and parasympathetic drugs have been shown to alter the tracheobronchial clearance rate of inert insoluble particles. The mucus on the lining layer of the conducting airways is propelled toward the larynx by ciliary activity. Thus, toxic agents could affect mucociliary clearance by altering the amount or quality of mucus produced within the respiratory tract, or by directly affecting respiratory tract ciliated cells. The current investigation is aimed at developing protocols to measure tracheobronchial mucus synthesis, secretion and transport rates *in vivo* in the rabbit.

The mucous lining layer contains a complex mixture of proteins and glycoproteins. One class of glycoproteins in the lining layer fluid, the mucous glycoproteins, is thought to be predominantly responsible for the viscoelastic characteristics necessary for the movement of mucus by respiratory tract cilia. Mucous glycoproteins differ from other glycoproteins in tracheobronchial secretions in their high molecular weight, high carbohydrate content and high content of fucose (6-deoxy-L-galactose). In the current study, fucose is used as a marker for mucous glycoproteins.

A sensitive analytical technique for fucose has been developed. The limit of sensitivity of this method (5×10^{-13} moles fucose) is approximately 2000-fold lower than those of previously published techniques. The efficiency of bronchopulmonary lavage in removing the mucous lining layer and the biochemical characteristics of lavaged materials are being examined. Mucus from lavage fluid is purified by ultrafiltration and fractional ethanol precipitation, a procedure which separates glycoproteins on the basis of their fractional carbohydrate content.

Preliminary experiments indicate that successive lavages remove progressively less macromolecular carbohydrate, fucose and protein. In crude lavage, macromolecular fucose accounts for approximately 4% of the total macromolecular carbohydrate. Fractional ethanol precipitation of lavage fluid results in a glycoprotein fraction in which the fucose content ranges between 7.0 and 9.7% of the total carbohydrate. Gallagher and Kent (*Biochem. J. 148*:187, 1975) have reported the fucose content of mucous glycoproteins synthesized by rabbit trachea *in vitro* to be approximately 8% of the total carbohydrate. Experiments are currently underway to determine the inter-rabbit variability in lung mucous glycoprotein content and the effect of parasympathomimetic drugs on the amount of lavagable mucus.

To examine the effects of environmental pollutants on the tracheobronchial mucociliary clearance system, the incorporation of ^{3}H-fucose into mucous glycoproteins and the absolute amount of mucous glycoproteins, as measured by fucose content, will be determined in mucus collected at the level of the larynx. Mucus removed by bronchopulmonary lavage and any mucus remaining in the respiratory tract after lavage will also be examined. These data will provide information on the amount of mucus transported to the level of the larynx over known time periods, the amount of mucus secreted into the airway lumen and its secretion rate, and the synthesis rate of mucous glycoproteins in the respiratory tract as a whole. These methods may provide a means of determining the mechanism by which toxic agents induce alteration in tracheobronchial mucociliary clearance rates.

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4. Alveolar Particle Retention, Clearance, and Translocation

The clearance and translocation of particles deposited in the non-ciliated portions of the lung have not been well characterized. Long-term observations of alveolar clearance based upon radiotracer measurements are scarce, and some of them may not accurately reflect the clearance of non-radioactive material. Therefore, a study of long-term pulmonary clearance has been begun using both inert and cytotoxic particles, to increase our understanding of the way dusts from occupational exposure are handled. In this study, *in vivo* dust retention and clearance measurements are being made using both radiotracer and magnetic methods in rabbits.

a. *In Vivo* Measurements of Dust Retention

Inhaled magnetite ($FeO \cdot Fe_2O_3$) dust particles may be detected magnetically by exposing the thorax of a subject that has inhaled the dust to an external magnetizing field. The field aligns the intrinsically magnetic magnetite particles within the lung, and when the external field is removed, the remanent magnetic field induced may be detected with a sensitive magnetometer. The magnitude of the remanent field is related to the concentration of magnetite (provided that the distribution remains constant), but, the aligned state is disturbed by respiratory motion. Consequently, the remanent magnetic field decays in a phenomenon termed "relaxation". The magnetic method uniquely detects the presence of magnetic particles rather than other forms of iron, which may occur by dissolution or conversion of magnetic iron to another compound, or dissolved magnetite, which loses its ferrimagnetic properties. Previous *in vivo* work in this laboratory using donkeys and neutron-activated magnetite (^{59}Fe), has confirmed that the decrease in remanent field for successive magnetizations and measurements is due to clearance rather than chemical conversion of magnetite.

An apparatus for magnetic clearance studies in rabbits has been constructed which consists of a fluidized-bed aerosol generator, a soft-iron core water-cooled electromagnet for localized-field magnetizations, and a precision non-magnetic scanning table with a fluxgate magnetometer. The fluidized-bed aerosol generator uses pre-classified dust of 2-4 μm MMAD to produce a magnetite aerosol at concentrations up to 250 mg/m^3 with a flow rate of 0.04 m^3/sec. Generation of an intrinsically magnetic aerosol is difficult since collisions between magnetite particles result in the formation of clusters and chains which increase effective particle size, decrease generation efficiency, and broaden the particle size spectrum.

The fluidized-bed generator is built into a shielded, instrumented cart which has a self-contained glove box for the safe transfer of neutron-activated magnetite from its shipping container to the generator.

The water-cooled electromagnet was designed for localized-field magnetopneumography, a procedure which maximizes sensitivity and permits direct quantitative calculations from remanent fields. The magnet generates a field of 0.55 Tesla at the pole face, dissipates 12 KW of power from a bank of storage batteries, and maintains an operational temperature below 40°C.

A precision non-magnetic restrainer has been built that provides rabbit placement that is reproducible to better than 5 mm in the plane of the skin surface and 1 mm in depth. Such accurate rabbit positioning is essential because remanent fields change rapidly with distance (r^{-3} for point-dipole sources).

The scanning table for the magnetic measurements was built of non-magnetic glass-epoxy and lumber, and the rabbit restrainer is fixed to the table by nylon pin guides. The fluxgate magnetometer sensors are mounted in gradiometer (opposed) configuration on a movable calibrated arm. By making measurements at different arm settings, the magnetite in the rabbit thorax can be mapped.

The radionuclide measurements are made with a pair of collimated scintillation detectors. The 3" x 3" NaI(Tl) crystals have an efficiency of approximately 10% for 1.1 meV photons. The collimators are 3/8" diameter holes in the 4" lead shielding about the detectors.

Initial observations made using this apparatus have been successful, and for the first time, clearance corresponding to the tracheobronchial phase has been observed magnetically. A $t_{\frac{1}{2}}$ of approximately 2.5 hours was seen following inhalation of magnetite aerosol. This value may not accurately reflect normal tracheobronchial clearance, since the aerosol was delivered by an endotracheal tube, which may have denuded the tracheal epithelium of cilia. Measurements made using the previous uniform-field method for magnetization did not show any "clearance" for the first two to three days, probably because the remanent fields resulting from

uniform-field magnetization are highly dependent upon magnetite distribution. The translocation of pulmonary magnetite during the first few days appears to distort the remanent fields.

These preliminary data provide support for the hypothesis that the localized-field magnetization method is less sensitive to distribution of magnetite in the lung and more sensitive to the concentration. The measured sensitivity (using phantom lung models) is 5 ng/cm^3/pt. The lower limit of sensitivity of the fluxgate magnetometer is ∿100 pt, corresponding to a sensitivity of 0.5 μg/cm^3 magnetite. The alveolar phase of clearance, observed from the second to the thirtieth day post-inhalation, had a $t_{\frac{1}{2}}$ of 9.5 to 12 days. This same phase observed in donkeys (using the uniform-field method) was 19 to 52 days.

Magnetic relaxation time is theoretically related to the rotational freedom of the magnetite particles in the lung. Thus, those particles bound within connective tissue should exhibit a slower relaxation than those suspended in mucus or free in the alveoli. Observed relaxation was very rapid ($t_{\frac{1}{2}}$ ∿2.5 minutes) immediately after magnetization. At 45 minutes after magnetization, the slope had changed to give a $t_{\frac{1}{2}}$ of ∿3 hours. These observations did not vary significantly over the first month of measurements. The relaxation curve suggests a "continuum" of particles ranging from tightly bound to free. Confirmation of this finding will be made using serial morphological and histological measurements.

The effects of inhalation exposures of rabbits to cytotoxic and inert dust upon lung clearance of particles by pulmonary alveolar macrophages (PAM) are being studied. For this study coal dust represents a cytotoxic dust, and magnetite an inert dust. The rabbits are exposed to either a low (10 mg/m^3) or a high (250 mg/m^3) concentration of dust in the respirable size range ($\leq$ 3.5 μm MMAD) for 1-2 hours.

b. Pulmonary Macrophage Assays

Before the animal exposures began, a considerable amount of work was performed in methods development and standardization. An *in vitro* assay system was developed to assess alveolar macrophage movement after dust exposure. This assay involves seeding macrophages on a surface that is densely coated with latex particles. With time, the macrophages engulf and ingest latex particles, leaving behind a cleared path. These particle-free tracks then provide a record of the cells' migratory pattern which can be viewed by low power microscopy. Increased migratory activity is seen when chemotactic factors are introduced into this system. Optimum cell movement was obtained at concentrations of 10^{-5} to 10^{-6}M N-formyl-L-methionyl-L-phenylalanine. These are the same concentrations reported in the literature when the traditional cell movement assays are employed. Compared to other movement assays, smaller numbers of cells are required. These standardization procedures were used to verify this technique's applicability to the assessment of alveolar macrophage movement.

The biological retention half-time of coal dust placed in simulated lung fluid was also investigated in studies where differences in the trace metal content of coal before and after exposure to lung fluid were used to determine changes in coal dust composition. Trace metal analysis, measured by nitric acid digestion, indicated that, in the coal dust sample, iron was the most abundant trace metal, with a concentration of 1.0 mg/g.

c. Localized-Field Magnetopneumography (LMPG)

A mathematical model has been developed to quantitate the magnetic clearance measurements. With this model, LMPG has been developed which may permit three-dimensional magnetic imaging of magnetite within the body.

The model is partitioned into three steps. First, the magnetizing field strength is computed at regular intervals within the thorax. Next, these values are used to calculate percentage of magnetic saturation for magentite in each location. Finally, the contribution of each of these "cells" to the total magnetic field seen by the magnetometer is predicted using the magnetic reciprocity theorem. This path constitutes the forward solution. Each "cell" within the thorax is represented by a sensitivity vector (S) which yields the remanent field (B) per unit magnetite mass (M). For all field measurements and "cells", the "forward" solution, in matrix form is $B = MS$. The magnetite at each cell may be solved by the matrix inversion $M = BS^{-1}$. Computer modelling of this solution demonstrates that crude three-dimensional magnetic images may be constructed from remanent field observations. This model will be tested using phantom lungs and rabbits.

Findings to date indicate that a solution for 27 "cells" in the rabbit chest (where the thorax is divided into a 3 x 3 x 3 array of masses) is feasible. The solution is strongly dependent upon the geometry of the magnetizing coil and the magnetometer.

d. Magnetopneumographic Observation of Occupational Lung Dust

In a collaborative effort with the Pulmonary Division of the Hahnemann Medical College and Hospital (Philadelphia), the pulmonary magnetic dust content in arc-welders, coal workers, machinists, asbestos sprayers, and control subjects has been measured *in vivo*. The respirable dust in workers from all of these occupations has a magnetic component. Using a sensitive super-conducting magnetometer, a pattern of central (hilar) and pleural accumulation of dust has been shown to result from chronic occupational dust exposure. The appearance of dust in these regions, which are rich in lymphatics, suggests the pattern of translocation of dust in man. Further cross-sectional studies are planned to relate exposure, pulmonary mechanics, and health factors to occupational dust accumulation.

Sponsor: National Institute for Occupational Safety and Health, Grant No. OH-00678

Principal Investigator: M. Lippmann, Ph.D.

Co-Investigators: S. Robinson, Ph.D., S. Williamson, Ph.D. (Physics), R.B. Schlesinger, Ph.D. and R.E. Albert, M.D.

Staff: H.H. Cheng, Ph.D., M.A. Leyko, M.S. and K. Barbee, B.S.

Publications:

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E. EPIDEMIOLOGY, BIOSTATISTICS AND BIOMATHEMATICS

1. Mortality Study of Occupational Exposure to Halo Ethers

The alkylating compound chloromethyl methyl ether (CMME), together with bis(chloromethyl)ether (BCME), which is often a contaminant in CMME, has been used extensively as a cross-linking agent in the manufacture of a variety of chemicals. Both agents were shown to be potent carcinogens in skin applications and inhalation studies carried out by investigators at this Institute. Epidemiological studies have also shown the compounds to be carcinogenic.

An industry-wide epidemiological study of the consequences of occupational exposure to CMME was conducted in 1972, for an evaluation of human carcinogenicity. The results of this retrospective survey of mortality data from 1948-1972 on a total cohort of about 10,600 workers (1800 CMME-exposed, 8800 control), clearly indicated that CMME-exposed workers had an increased risk of developing respiratory cancer (about 2.5 times that of the control population).

The largest of the six firms studied provided detailed information on job classification and exposure duration. This permitted qualitative estimation of the relative intensities of maximum exposures. Workers classified as subject to heavy exposure for over five years had a risk of lung cancer death nearly 24 times the control group. Furthermore, among those with heavy exposure, a short latent period between exposure onset and respiratory cancer was observed, and eight lung cancer deaths occurred before age 45. An exposure-response analysis yielded highly significant trends with respect to both duration of exposure and integral exposure (cumulative duration times intensity), although the magnitude of risk at low exposure levels was unclear. An analysis of exposure level vs. the induction-latency period showed significantly longer latencies at lower exposure levels. An analysis of lung cancer incidence by interval of time since the individual's last CMME exposure showed that excess lung cancer risk had not diminished even 25 years after exposure ceased.

Because of the longer latencies and the uncertainty about the magnitude of risk at lower doses, the mortality follow-up of the original cohort is being extended another seven years, and a seventh CMME producing firm has been included for mortality surveillance from 1955 through 1979. A classification scheme will be developed for ranking the magnitude of CMME exposure at this firm. Company records and information obtained from the Social Security Administration are being used to identify deaths at all firms. Death certificates will be obtained, and hospital and pathology records will be sought for cancer deaths of interest.

Sponsor: National Institute for Occupational Safety and Health, Grant No. OH-00932.

Principal Investigator: B.S. Pasternack, Ph.D.

Co-Investigators: R.E. Shore, Ph.D., R.E. Albert, M.D. and M. Lippmann, Ph.D.

Staff: K. Wales, M.S. and J. Garcia

Publications:

Albert, R., B. Pasternack, R. Shore, M. Lippmann, N. Nelson and B. Ferris. Mortality patterns among workers exposed to chloromethyl ethers -- A preliminary report. *Environ. Health Perspect. 11*:209, 1975.

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Pasternack, B. and R. Shore. Lung cancer following exposure to chloromethyl ethers. In: *Proc. International Conference on Critical Current Issues in Environmental Health Hazards*. Tel Aviv, Israel, March 1979. In Press.

2. Cancer Mortality after Arsenic Exposure

Although most studies of populations exposed to arsenic have shown it to be carcinogenic, little historical information has been available on exposure levels, which could be used to develop absolute risk estimates or to examine dose-response relationships.

About 1300 patients treated for syphillis with arsenical drugs at Bellevue Hospital between 1940 and 1945 and 1300 treated with penicillin during 1945-48 have been followed to determine their mortality experience. The age, sex, and race distributions of these two groups were comparable, and accurate exposure information was available. The doses of arsenic were low but ranged over two orders of magnitude (0.03 to 3.0 g). Deaths were identified through hospital records and the Social Security system. Thus far, information has been obtained on about 475 deaths.

Analyses were conducted to control for sex, age, and the time interval since treatment. The analyses were performed for arsenic exposed individuals and controls, and for the dose-response relationship. The only significant dose-response trend seen was for genitourinary cancers, but this was not conclusive because of the small numbers. There were also suggestive, but not significant, excesses of respiratory cancer, brain cancer, and multiple myeloma in the arsenic-exposed group.

Sponsor: Institute of Environmental Medicine

Principal Investigators: B.S. Pasternack, Ph.D., R.E. Albert, M.D. and R.E. Shore, Ph.D.

Staff: K. Koenig, B.A.

3. Effects of Cutting Oil Exposures on Bearing Workers

Cutting oils have been shown to contain known carcinogens including benzo(a)pyrene in natural and soluble cutting oils, and N-nitrosodiethanolamine in synthetic and semi-synthetic cutting oils. Epidemiological studies of workers exposed to cutting oils, however, have not yielded consistent results. An occupational cohort has been identified which has had heavy exposure to cutting oils in the bearing industry, and planning has begun on an historical cohort study in cooperation with the United Automobile Workers (UAW) Union, to investigate subsequent cancer mortality.

Members of the cohort will be an estimated 11,000 union members employed for five or more years between 1950 and 1980 at a large bearing plant in central Connecticut. Current vital status will be determined from UAW records, the Social Security Administration, and from other sources, and exposure to cutting oils will be determined from job histories. The relationship between the degree and duration of exposure and cancer mortality will then be examined by computing standardized mortality ratios by cause and cumulative cutting oil exposure, using United States mortality rates by age, race, sex, and calendar year as a standard. Cutting oil exposures in respiratory and stomach cancer deaths will be further investigated by conducting embedded case-control studies, using a random sample of the rest of the cohort as controls. More detailed work histories, ethnicity, and smoking histories will be sought for subjects in this part of the research project.

Sponsor: Institute of Environmental Medicine

Principal Investigators: M. Marmor, Ph.D., B.S. Pasternack, Ph.D. and N. Nelson, Ph.D.

Co-Investigators: R.E. Shore, Ph.D., M. Lippmann, Ph.D. and M. Schwartz M.D., Ph.D.

4. Sequelae to Childhood Lead Exposures

Previous studies have suggested that neuropsychological function is affected by childhood lead exposures. Deficits of 4 points in performance

on standardized tests of intelligence, for example, have been observed by Needleman *et al.* in lead exposed children, when compared with less-exposed controls (*N. Engl. J. Med. 300*:689, 1975). In such studies, the comparability of exposed and control subjects in variables other than lead exposure, such as in the quality of the home environment, is critical because such variables may be correlated both with intelligence and with the probability of being exposed to lead or practicing pica. The present investigation is designed to minimize differences between exposed and control subjects in non-lead variables by locating and testing twins with discordant histories of lead exposure.

Subjects for this study were obtained from the records of the New York City Department of Health, Bureau of Lead Poisoning Control. Index twins were defined to be lead poisoning cases on the basis of blood lead levels measured between 1970 and 1979 and were between 6 and 15 years of age at recall in 1979-1980. Their co-twins, on the other hand, were never known to be lead poisoning cases and had maximum blood lead levels at least 10 μg/dl less than their lead poisoned siblings. Sixty-five apparently discordant twin pairs were located, of whom 48 have been tested with the Bender Gestalt Test for Young Children, the Wechsler Intelligence Scale for Children - Revised (WISC-R), tests of reaction time, finger-wrist tapping speed, the Sentence Repitition Test, the Seashore Rhythm Test, and the Children's Behavior Inventory. Physical examinations and parental interviews were administered, and school and medical records are being sought.

A preliminary analysis of the first 20 twin pairs has been conducted. Three twin pairs had to be discarded from categorical data analysis because they did not meet the criteria for discordance. Scores of the remaining 17 twin pairs, however, suggested that lead exposure was associated with a deficit in the total WISC-R score of about 6 points ($p = 0.06$) and with a similar deficit of 6 points on the performance subtest of the WISC-R ($p = 0.04$). Neither the verbal portion of the WISC-R nor the other elements of the test battery show significant differences at this point. Final statistical analysis of the data from 42 discordant and 6 concordant twin pairs is under way, using both categorical and regression techniques. Hair and tooth samples are being analyzed for lead content as a further check on discordance in lead poisoning histories within twin pairs.

Sponsor: National Institute of Environmental Health Sciences, Grant No. ES 02620

Principal Investigator: M. Marmor, Ph.D.

Co-Investigators: B.S. Pasternack, Ph.D. and D. Goldberg, M.D. (Bureau of Child Health, New York City Department of Health)

Publication:

Marmor, M. and D. Goldberg. Sequelae to childhood lead exposure: A twin study. 108th Annual Meeting of the American Public Health Association, Detroit, October 19-23, Abstract, 1980.

5. Sequelae of Childhood Lead Exposure: A Twin Study

Studies on the effects of moderately elevated blood levels of lead have been carried out both in children and in laboratory animals. In clinical studies of children that have been carried out by neurologists, pediatricians, and clinical psychologists, it has been found that blood levels of lead in the range of 30-80 µg per 100 ml cause subtle behavioral and neurological deficits. In animal studies, other behavioral and neurological responses to lead have been shown. Extrapolation of the results from animal studies to human beings, however, has proven difficult, since there is a large gap in psychological and intellectual performance between the two groups.

To bridge the performance gap, studies have been initiated that will measure performance impairment in lead-exposed children. The methods being used will also be applied to people from a wide variety of cultural and socio-economic backgrounds, and to experimental animals. Through these studies, a common index of nervous system dysfunction will be obtained which will allow meaningful comparisons to be made between laboratory animals and human beings.

The subjects studied thus far have been 8 twin pairs, ranging from 6 to 15 years of age, who had previously received physical and clinical psychological examinations in a study by Cornell University and the New York City Bureau of Lead Poisoning Control. The twins were selected for the study if they had discordant blood lead concentrations. By relating neuropsychological performance to blood lead within each pair of twins, it was possible to reduce the variability that has been encountered in other studies of unrelated children. Data have been recorded on motor activity, hand steadiness, time discrimination, learning and memory of a visual discrimination, visual acuity and tactile sensitivity. Preliminary analyses indicate that there is a significant deficit in visual learning in the twins that previously had elevated blood lead levels. Additional data analyses and experiments are under way to further define the degree of the deficit.

Sponsor: Institute of Environmental Medicine and a subcontract from Cornell University

Principal Investigator: H.L. Evans, Ph.D.

Co-Investigators: S.A. Daniel, Ph.D., J.J. Teal, Ph.D. and M. Marmor, Ph.D.

Staff: A.M. Dempster, B.A.

6. Risk Factors for Minimal Breast Cancer in Initially Asymptomatic Women

The purpose of this case-control study is the assessment of risk factors associated with minimal breast cancer in initially asymptomatic women, to determine whether minimal breast cancer is an early manifestation of clincial breast cancer or is, alternatively, fundamentally different in character.

One thousand, three hundred and ninety women diagnosed with breast cancer between 1969 and 1979 have been identified at the Guttman Breast Diagnostic Institute, along with 2465 controls without breast cancer who were randomly selected from the same screening population. Collection of demographic and screening data is essentially complete for those 3855 participants. The 1390 positive cases are being further classified by means of pathologic review of slides and other materials requested from 131 hospitals (primarily in the New York metropolitan area). This review is being performed independently by two teams of pathologists. Pathological material which had been requested for 756 women, has been received for 573, and it has been reviewed at NYU and at St. Barnabas Medical Center. A review of mammography films has been requested for 746 women and, of these, 447 have been completed.

Specifically-designed computer programs are being used to edit the data to establish the internal consistency of each data record, to assess the completeness of each participant's data file, and to coordinate the various data records for each participant. Error messages produced by the program identify inconsistencies which are resolved by examination of the participants' files both at New York Unviversity and at the Guttman Institute.

Once the editing of demographic and screening data is complete, a risk factor analysis will be performed that should result in relative risk estimates within the range found at other screening centers. Risk factors studied include age, age at menarche, age at first birth, age at menopause, race, obesity, previous breast disease, family history of breast cancer, and use of oral contraceptives. Subsequently, risk factors associated with tumor aggressiveness will be assessed by comparing separately (in increasing order of aggressiveness) prevalent, incident, and interval cases with controls. Upon completion of pathology review and concomitant data editing, cases thus classified as minimal or clinical will be compared separately with controls, to determine whether cases which differ in tumor invasiveness are characterized by different risk factor distributions.

Sponsor: National Cancer Institute, Contract No. NO1-CB-74103

Principal Investigator: B.S. Pasternack, Ph.D.

Co-Investigators: R.V.P. Hutter, M.D. (St. Barnabas Medical Center, Livingston, NJ) and P. Strax, M.D. (Guttman Breast Diagnostic Institute, New York, NY)

Staff: R. Schinella, M.D., E. Fazzini, M.D., N. Dubin, Ph.D., R. Shore, Ph.D. M. Moseson, M.P.H., Z. Herzberg, B.S., S. Brown, B.S., B. McLaughlin, B.S., D. Friedman, B.S. and R. Collazo

7. Hair Dye Use and Breast Cancer

The possibility that hair dyes may be carcinogenic was suggested when Ames found a number of dyes to be mutagenic in a bacterial test system. However, the results of animal and human studies on the carcinogenicity of hair dyes have been conflicting. In a previous study in this laboratory, hair dye use was found to exhibit a dose-response relationship with breast cancer when the latent period was taken into account. Since the number of women in this earlier study was fairly small, a larger study is under way to clarify the relationship between hair dye use and breast cancer. Since approximately 25 million women in the U.S. dye their hair, this issue has important public health implications.

The study group consists of approximately 500 women in whom breast cancer was or will be detected at the Guttman Breast Diagnostic Institute (GBDI) between 1977 and 1982, and about 1000 randomly selected (control) women without breast cancer undergoing screening at GBDI during the same period. The probability of developing breast cancer and the probability of using hair dye both increase with age, and therefore, a strictly random selection process produces controls whose average age is lower than that of the cases. To eliminate these undesirable age differences, the number of controls in each age group is adjusted to the number of cases, by randomly selecting twice as many controls from each 5-year age groups as there are cases in that group. This process involves projections of the numbers and age distributions of new cases and controls to be acquired in 1980-82, based on the experience of 1977-79.

Information from subjects on breast cancer risk factors and hair dye use is being obtained through a structured telephone interview, with home interviews as necessary. The interviewer is kept blind as to the case or control status of the respondent until after the questions on hair dye use have been asked. The interview schedule was refined by a pilot test on 40 women last screened at GBDI in 1976. The questionnaire includes items on pregnancy history, breast feeding, menstrual history and menopause, height and weight, family history of breast cancer,

respondant's history of breast disease, uterine or ovarian abnormalities, surgery or x-irradiation, other medical conditions including cancer, liver disorders, diabetes and hypertension, use of birth control pills, menopausal estrogens, thyroid medications and other hormonal medications, other chronic medications, tobacco and alcohol use, and sociodemographic factors including age, marital status, education, family income, religion, ethnicity, and residence. Questionnaire items pertaining to the hair include natural color, presence and age of greying, and use of permanents. Hair dye questions obtain a temporal history of use by color, brand, and type of dye (permanent, semi-permanent, color rinse, bleach, metallic dye, or vegetable extract).

Hair dye use among the cases and controls will be compared while controlling for other breast cancer risk factors and allowing for a latency period. The dose-response relationship between hair dye use and breast cancer will be investigated, and interactions between hair dye exposure and other risk factors will be assessed.

Sponsor: National Institute of Environmental Health Sciences, Grant No. ES 02436

Principal Investigator: B.S. Pasternack, Ph.D.

Co-Investigators: R.E. Shore, Ph.D. and P. Strax (Guttman Breast Diagnostic Institute, New York, NY)

Staff: N. Dubin, Ph.D., K. Koenig, B.A., N. Sloan, M.P.H., M. Moseson, M.P.H., S. Hecht, M.P.H., M. Eris and L. Yee

8. Epidemiology of Malignant Melanoma

The objective of this study is to determine the degree of association between the occurrence of malignant melanoma and possible risk factors, including some of those which have not received quantitative analysis previously. To accomplish this, data collected at the New York University Skin and Cancer Unit since 1972 will be analyzed.

A multivariate risk factor score will be developed to quantify the association between the occurrence of malignant melanoma and such classic risk factors as age, sex, race, ethnic origin, complexion, ability to tan, hair color, eye color, and sun exposure. In addition, speculative variables such as use of birth control pills and other contraceptives, age at menarche, age at menopause, numbers of moles, tendency to freckle, use of phenothiazines and other drugs, tobacco and alcohol consumption, dietary factors (caffeine, polyunsaturated fats, sugar substitutes, and vitamin A), a lengthy checklist of chemical exposures (including occupational), fluorescent and ultraviolet light exposure, and religion will

be included. Whenever possible, quantitative variables will be emphasized. For example, with respect to sun exposure, interviews are designed to elicit the actual hours of exposure per day during various time periods in the previous twenty years.

The total data base will consist of 1250 cases and 1100 controls. Of the total cases, 801 were interviewed by the NYU Melanoma Cooperative Group during 1972-1979, prior to the start of this study. Data for these have been transferred onto revised data forms to make them compatible with the computerization of data to be collected prospectively as part of this study. They have been keypunched, and edited on-line, using the CDC 6600 computer at the NYU Courant Institute of Mathematical Sciences. Since April, 1979, 244 additional cases and 410 controls (randomly chosen from Skin and Cancer Unit patients without melanoma or other neoplastic conditions) have been interviewed. Based on current accession rates, the remaining 205 cases should be available by October, 1981, and the remaining 690 controls should also be available at that time.

Sponsor: National Institute for Occupational Safety and Health, Grant No. R01-OH00915

Principal Investigator: B.S. Pasternack, Ph.D.

Co-Investigator: A.W. Kopf, M.D (Department of Dermatology)

Staff: N. Dubin, Ph.D., R. Shore, Ph.D. P. Hennessey, M.D., R. Friedman, M.D., M. Moseson, M.P.H., E. Greenwald, M.P.H., Z. Herzberg, B.S., S. Brown, B.S., F. Viau, R. Collazo and M. Eris

Publications:

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9. Follow-up Study of Irradiated Tinea Capitis Cases

For over 50 years, the most effective treatment of ringworm of the scalp (Tinea capitis) was complete scalp epilation by x-ray therapy. A group of about 2,200 children who had received this treatment at New York University Hospital between 1940 and 1959 are being studied, along with about 1,400 children (controls) who received only topical medications for Tinea capitis during the same period.

The irradiated and control subjects have been followed an average of 26 years (with a maximum of 35 years) since their ringworm treatment, by health questionnaires, with medical and pathological verification of pertinent conditions. The response rates have been high (90-95%), and the groups are comparable on important demographic variables such as race, sex, socio-economic status, and age.

Eight brain tumors have been found in the irradiated group (1 glioma, 1 hemangioblastoma, 2 astrocytomas, 2 meningiomas, and 2 acoustic neuromas), while there have been none among the controls. A dosimetric study showed that doses to the brain ranged from about 175 rads at the apical surface to 70 rads at the base. Based on age- and sex-specific rates for both benign and malignant brain tumors from the Connecticut tumor registry, about 1.4 tumors would have been expected in the irradiated group. This yields a brain tumor risk of about $1.0/10^6$ persons/yr/rad.

Nine thyroid adenomas have been diagnosed in the irradiated group and none among the controls. No thyroid cancers have been observed yet, however. Since the average dose to the thyroid was only 6 ± 2 rads, the data provide evidence for the sensitivity of the thyroid gland to even low doses of radiation. The risk per rad for thyroid adenomas in this study is comparable to that seen in other studies at higher doses, suggesting approximate linearity of the dose-response curve for thyroid adenomas.

Forty irradiated subjects had a total of 78 basal cell carcinomas and 1 squamous cell carcinoma on the irradiated skin of the head and neck, compared to 3 in the control group. Thirty skin cancers occurred on the scalp, and 49 appeared near the margins of the scalp or elsewhere on the head and neck. A detailed dosimetry has been performed to characterize the dose distribution on the head, and the risk per rad per cm^2 of skin proved to be about 5 times as great for skin with x-rays plus ultraviolet exposure as for the hair-covered scalp (with x-rays but little UV exposure). The fact that all 79 skin cancers were found among the 76% of the irradiated group who were whites, and none was found among the 24% blacks, also points toward UV exposure as an important potentiating factor.

A total of 220 irradiated and 91 control subjects were given a dermatologic examination. Of the 203 irradiated whites with no previous diagnosis of skin cancer, 10 (4.9%) were found to have basal cell carcinomas on the head or neck, indicating that the prevalence of skin cancers is substantial in the irradiated group. None was found among controls. The clinical examination also gave evidence of excessive generalized hair thinning in the irradiated group.

Sponsor: National Cancer Institute, Grant No. CA 17188

Principal Investigators: R.E. Albert, M.D. and R.E. Shore, Ph.D.

Co-Investigators: B.S. Pasternack, Ph.D., A. Friedhoff, M.D. (Department of Psychiatry), N. Harley, Ph.D. and M. Reed, M.D. (Department of Dermatology)

Staff: L. Yee and J. Garcia

Publications:

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Albert, R., F. Burns and R. Shore. Comparison of the incidence and time patterns of radiation-induced skin cancer in humans and rats. In: *Late Biological Effects of Ionizing Radiation, Volume 1*. International Atomic Energy Agency, Vienna, p. 499, 1978.

Shore, R.E., R.E. Albert and B.S. Pasternack. Long-term effects of x-ray therapy for ringworm of the scalp. *Amer. J. Epidemiol. 112*:442, Abstract, 1980.

10. Breast Cancer in Women Irradiated for Postpartum Mastitis

The purpose of this study is to estimate the magnitude of the breast cancer risk from ionizing radiation. This is of concern because the breast is exposed to radiation in medical screening and diagnostic procedures, and occupational exposures of women are increasing.

Approximately 570 women in the Rochester, NY area who received x-ray therapy for acute postpartum mastitis, a common inflammatory breast disease of childbirth., are being studied. A total of 990 women, consisting of unirradiated postpartum mastitis patients, and siblings of all the mastitis patients are serving as controls. With these controls, both familial/genetic factors and mastitis itself can be eliminated as causes of any excess cancers in the irradiated group.

Subjects have been followed for up to 35 years post-irradiation by mail or by interview questionnaire, with medical verification of all diseases of interest. A new survey of these groups, which will extend the observation an additional six years, has been completed. The data are being coded for analysis, but results are not yet available.

In the latest available data, there were 37 breast cancers in the irradiated group and 34 in the larger control group. After adjusting for age at irradiation and the interval since irradiation, a 2-fold increased risk was found in the irradiated group. The average breast tissue dose was 250 rads (with a range of 60 to 1300 rads). On an absolute risk basis, the radiation risk of breast cancer was $8/10^6$ women/year/rad. For the two-thirds of the women with only one breast irradiated, the increased incidence was limited to the irradiated breast.

The dose-response relationship was approximately linear to 400 rads, above which there was a downturn, apparently due to "cell killing" or other high-dose phenomena. Since these data were collected, they have been analyzed in parallel with two other major sets of data on radiation-induced breast cancer, the Japanese atomic bomb study and the Massachusetts multiple fluoroscopy study. In all of these studies, the dose-response curves were approximately linear, with an average cancer risk of $6.6/10^6$ women/year/rad.

Factors which might modify the carcinogenic response were also examined. Within the limits of a relatively small range of dose-fractionation, no fractionation effect could be detected. Over the age range of 20-45 years at irradiation, no variation in risk by age was found. Both pre-menopausal and post-menopausal breast cancer incidences were elevated in the irradiated group.

The possibility of synergism between radiation and other risk factors for breast cancer was examined. No synergism was found for family history of breast cancer, late parity, oral contraceptive use, or menopausal estrogen use. However, there were indications that there was extra radiation-related risk if the irradiation occurred at the first childbirth, or if cystic breast disease developed.

Sponsor: National Cancer Institute, Grant No. CA 21452 and Food and Drug Administration, Grant No. FD 00634-14 (Subcontracts from the University of Rochester)

Principal Investigator: R.E. Shore, Ph.D.

Co-Investigators: B.S. Pasternack, Ph.D., R.E. Albert, M.D. E. Woodard, M.D. (University of Rochester) and L. Hempelmann, M.D. (University of Rochester)

Staff: L. Yee

Publications:

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Shore, R. and B. Pasternack. Radiation risk of mammography: A reply. *Canadian Med. Assoc. J. 123*:97, 1980.

Land, C., J. Boice, R. Shore, J. Norman and M. Tokunaga. Breast cancer risk from low-dose exposures to ionizing radiation: Results of parallel analysis of three exposed populations of women. *J. Natl. Cancer Inst. 65*:353, 1980.

Shore, R., E. Woodard, L. Hempelmann and B. Pasternack. Synergism between radiation and other risk factors for breast cancer. *Preventive Med. 9*:815, 1980.

11. Thyroid Neoplasms Following Thymic Irradiation in Infancy

The purpose of this study is to quantitatively estimate the tumorigenic risk to the thyroid from x-irradiation. It is a collaborative effort with Drs. Hempelmann and Woodard of the University of Rochester to follow about 2,700 subjects given x-ray therapy to shrink allegedly enlarged thymus glands in infancy, and their 5,000 siblings (controls). The subjects have been followed for up to 40 years post-irradiation by mail or interview questionnaire, with medical verification of pertinent conditions.

The thyroid doses received depended on the number of x-ray treatments and on whether the thyroid was inside the primary beam. The doses ranged from 5 to 1300 rads, with an average of about 120 rads. In the irradiated group, 24 thyroid cancers and 52 adenomas were observed,

while there were no thyroid cancers and 6 adenomas in the control group. Analyses of the dose-response curve showed a strong linear component, with a slope of 3.8 ± 0.3 excess cancers/10^6 persons/year/rad. In addition, there was a smaller but significant dose-squared component to the curve, where the risk per rad was 2-3 times as great at high doses as at low doses. The thyroid adenoma dose-response curve also showed a strong linear component, with a slope of 4.5/10^6 persons/year/rad, but it had no dose-squared component.

Analyses of dose fractionation suggested a "sparing" effect of dose-fractionation for thyroid cancers, but not for thyroid adenomas. Thus, linear extrapolation to low doses or to low dose-rates appears to provide a reasonable guide for thyroid radiation protection.

Questions of tumor latency and host susceptibility were also explored. For both thyroid cancer and thyroid adenomas, the evidence indicates that radiation-induced excess tumor incidence is sustained for at least 40 years post-irradiation (the maximum length of follow-up). The data also showed that both female and Jewish subjects had greater radiation induction of thyroid cancer than did their counterparts.

Another survey extending the observations for an additional seven years has been completed, and it is nearly ready for analysis.

Sponsor: U.S. Public Health Service, Grant No. RL 00078 (Subcontract from the University of Rochester)

Prinicpal Investigators: R.E. Shore, Ph.D. and B.S. Pasternack, Ph.D.

Co-Investigators: L.H. Hempelmann, M.D. and E. Woodard (University of Rochester)

Staff: J. Garcia and L. Yee

Publications:

Shore, R., L. Hempelmann, B. Pasternack and E. Woodard. Radiation- and host-factors in thyroid tumors following thymus irradiation. *Amer. J. Epidemiol. 108*:233, Abstract, 1978.

Shore, R.E., E. Woodard, B. Pasternack, L. Hempelmann. Radiation and host factors in human thyroid tumors following thymus irradiation. *Health Phys. 38*:451, 1980.

12. Cancer Mortality in Bergen County, NJ, 1962-1975

Publication of the *Atlas of Cancer Mortality for U.S. Counties, 1950-1969* caused a great deal of concern in counties shown by the Atlas

to have had high cancer mortality rates, relative to the United States as a whole. This laboratory has analyzed temporal trends of cancer mortality in Bergen County, a high-rate county located in northeastern New Jersey, by calculating age-adjusted cancer mortality rates by sex and site for Bergen County residents for the period 1962-1975. Mortality rates and time rates of change in mortality rates were compared with those in the United States as a whole.

The analysis showed that some of the relationships between Bergen County and United States cancer mortality rates which existed in 1950-1969 did not continue in more recent years. For example, the Atlas showed Bergen County to have a mortality rate for cancer of all sites combined among white males that was both significantly higher than the rate in the United States as a whole and in the highest decile of all county rates for the period 1950-1969. Similar excesses were shown for male and female respiratory cancer mortality rates. When more recent temporal data were considered, however, it was clear that the differences between Bergen County and the United States for each of these sites have consistently diminished until, in 1974-1975, the differences between the two populations were negligible. Graphs have been prepared for each of the major cancer sites by sex to illustrate these trends further, and the implications of this research for cancer control and for educational programs in Bergen County have been discussed with appropriate officials.

Sponsor: Institute of Environmental Medicine

Principal Investigators: M. Marmor, Ph.D. and B.S. Pasternack, Ph.D.

Publication:

Marmor, M., M. Sadow, K. Green and L. Samilow. Cancer mortality in Bergen County, NJ, 1962-1975. *Public Health Reports*. In Press.

13. Population Health Survey--Staten Island

The medical literature, both past and present, documents a finding of high age-adjusted respiratory cancer death rates on Staten Island. Staten Island has led the other Boroughs of New York City in these rates for at least half a century.

Although the quality of the air shed over Staten Island, with pollution from the industrial complexes on the New Jersey shores, has been implicated as a cause of the problem, important demographic data necessary to test this hypothesis have been lacking. No concrete information exists on the residents' smoking habits, length of residence, family history, and so on.

A survey document to acquire the necessary data base has been developed and field tested. A volunteer corps of interviewers has also been trained, and it is currently in the field. Approximately 30 interviews have been conducted thus far. The goal of the research is to conduct between 1000-1500 interviews each year for one to three years.

The sampling plan which has been developed is stratified according to the 10 health areas which comprise the borough, each of which has different age-adjusted rates.

Sponsor: Institute of Environmental Medicine

Principal Investigator: M.S. Schwartz, M.D., Ph.D.

Co-Investigator: M. Israel, M.A.

Publications:

Israel, M., J. Gibbons and T. Chen. Trends in respiratory cancer deaths in New York City 1960-1977. *N.Y.C. Dept. of Health, Office of Biostatistics*. New York, NY 10013, February, 1979.

Greenburg, L., F. Field, J.I. Reed and M. Glasser. Air pollution and cancer mortality study of Staten Island, New York. *Arch. Environ. Health 15*:356, 1967.

Meyers, J. Cancer death-rate variation in relation to combustion products of fuel, topography, and population. *NY Journal Med. 28*:365, 1927.

14. Effects of Exposure to Summertime Haze Episodes on the Health of Children

The primary objective of this study was to determine if exposure to summertime hazy air masses containing ozone, sulfuric acid mist and a variety of other primary and secondary air pollutants produce measurable health effects in healthy children.

An additional objective was to quantitatively characterize the summertime exposures of people in a small non-industrial city in Pennsylvania to air pollutants, especially to acidic aerosols, including sulfuric acid. Such aerosols are an important part of the characteristic hazy air masses that develop and travel within the northeastern quadrant of the United States many times during each summer.

Human exposures to acidic aerosols in the ambient air are known to occur, but their frequency and magnitude are not known. They may increase in future years if there is greater utilization of high-sulfur coal without effective stack scrubbing. The summer of 1980 was considered to be an ideal time to launch this study, since the U.S. Environmental

Protection Agency (EPA) was supporting a major study of Prolonged Elevated Pollution Episodes (PEPE's) for 4 weeks during the summer, in a program given the acronym EPA-PEPE-80. The studied region covers a 500 km^2 area which focuses primarily on the Ohio River Valley, extending westward to St. Louis and eastward into central Pennsylvania. Within this region, past experience indicates that 6 to 10 pollution haze episodes, each lasting from 3 to 5 days, can be expected to occur each summer. EPA-PEPE-80 combined the efforts of a group of governmental and univeristy researchers, and it coordinated a substantial network of sampling and other environmental measurement stations, including aircraft and satellites. This permitted the investigators to anticipate the occurrence and locations of haze episodes, and to construct pollution exposure maps for a variety of air pollutants during and after the passage of the hazy air masses.

The support team for EPA-PEPE-80 provided advance notice of the arrival of the hazy air, and good exposure estimates of pollutants of interest, such as O_3, $SO_4^=$, NH_4^+, NO_3^-, SO_2, NH_3, b_{scat}, etc., using data collected on-site and at State monitoring stations. Concentration data on H_2SO_4, a critically important component of the mixture with respect to the potential health effects which are the focus of this study, were required, and thus, this program included a sampling component specifically directed at generating reliable exposures estimates of H_2SO_4 for the population of interest. This included sampling with H_3PO_4-treated quartz fiber filters, with analysis of the strong acid (H^+) component of the aerosol. In addition, in a cooperative effort by Drs. Husar and Lioy, a flame photometric detector was operated continuously to provide a continuous indication of the concentration of aerosol sulfur. It was supplemented by *in situ* thermal analysis, which provided data on the concentrations of H_2SO_4 and its ammonium salts.

The study focused on pre-adolescent children for the following reasons:

a. Complications due to cigarette smoke exposures can be avoided more readily than in older groups.

b. Cooperation and participation in the series of interviews and tests required can more readily be achieved than with older groups.

c. Children, especially those engaging in supervised summer recreational programs, will be out-of-doors for a larger portion of the time than will older groups, and outdoor concentrations of O_3 and H_2SO_4 should be greater than those indoors.

d. Any effects observable in children may have significance for the pathogenesis of chronic respiratory tract diseases and functional decrements, later in life.

The location of the health effects study population in Indiana, PA was almost ideal for this investigation because:

a. It was downwind of the major sources in the PEPE study region, providing a unique opportunity for the collection of a large range of both essential and accessory environmental data.

b. It was within that part of the U.S. which normally has the highest concentrations of O_3 and $SO_4^=$ during the summer haze episodes.

c. It was within a region which is virtually unaffected by local sources of anthropogenic pollutants. It was initially selected by Speizer and co-workers as the control population for their study of the impact of pollution from the complex of western Pennsylvania mine-mouth coal-burning electric-utility power plants. Being upwind of these plants in a sparsely populated forested area ensures that the population's dominant air pollution exposure is due to long-range transport from distant upwind sources. Thus, if these children were to exhibit any measurable health effects during or after the passage of the hazy air masses, there would be a minimum of alternative or secondary casual factors to complicate the interpretation of the impact of the transported pollutants.

d. A population of approximately 85 of the 120 children who enrolled for the June 30 to July 11, 1980 special summer program sponsored by the Presbyterian Church and the YMCA in Indiana, PA participated in the health effects studies.

The health effects analyses were performed under the supervision of Drs. Speizer and Fife of Harvard Medical School. Two medical students from NYU Medical Center with prior research experience in the Institute of Environmental Medicine were engaged in the testing of the children for the study.

Parents of the children registered for the June 30 to July 11, 1980 combined Presbyterian Church-YMCA summer program at the time of registration (April-June) received questionnaires asking for background respiratory disease histories of the children and household characteristics. Permission to participate in the physiological measurements was obtained for 85 children. The Church-YMCA program was operated so that the children spent every afternoon engaged in varied activities at the YMCA. As many as possible of the 85 available children were studied every afternoon during the two-week period. On the first occasion, the height in stocking feet and weight of each child were determined. The children then performed maximum respiratory maneuvers on a Collins water-filled portable recording spirometer. In addition, each child was assigned a uniquely identified mini-Wright Peak Flow Meter. On a daily basis, each child repeated the mini-Wright Peak Flow measurement, and

performed the full spirometer procedures whenever time permitted. These tests were done with the child sitting without nose clip, and the temperature of the recording bell was recorded so that all values can eventually be converted to BTPS.

The sampling program has been completed, and an analysis of ambient air quality and lung function data is being conducted by NYU personnel. Analysis of the health effects data will be based upon the differences in pulmonary function measurements made only on "clean" and "dirty" days.

Among the potential difficulties with this study, was the recognition that there might be no PEPE during the study period. This in fact was the case and attempts were therefore made to extend the duration of the study with the cooperation of all of the parties involved. Personnel remained in the field for part of the week of July 14 in this study extension, but no clearcut PEPE occurred then either.

There were hourly average ozone concentrations during the study period as high as 240 $\mu g/m^3$, and 6 hr TSP concentrations as high as 100 $\mu m/m^3$. However, there was no measurable H_2SO_4 above the 0.1 $\mu g/m^3$ limit of detection. While the analyses under way may still show some association between pollutant concentrations and respiratory mechanical function in the children, it is more likely that this past summer's study will provide a baseline period for a similar study in a future summer, when more typical meteorological patterns may prevail.

Sponsor: U.S. Environmental Protection Agency, Grant No. R 807723

Principal Investigator: M. Lippmann, Ph.D.

Co-Investigators: P. Lioy, Ph.D., B. Pasternack, Ph.D., N. Nelson, Ph.D. F.E. Speizer, M.D. (Harvard Medical School), and D. Fife, M.D. (Harvard Medical School)

Staff: K. Green, Ph.D., M. Morandi, B.S., D. Baxter, B.S. and G. Schwartz, M.S.

15. Risks Associated with Infant Transport

The objective of this study is to evaluate the effect of neonatal transportation of high-risk infants on mortality. The study design will enable a comparison of transported and non-transported newborns, matched for hospital of birth, weight, gestational age, and severity of condition.

The principal investigator is the director of the Infant Transport Service of New York City, the only service of its kind officially sanctioned by the New York City Department of Health. During the course of the year, this service transports approximately 1100 newborn infants, approximately 85% of those moved in New York City, to hospitals with more specialized facilities. It serves approximately 58 acute care facilities that maintain maternity and newborn services which together accomodate approximately 110,000 live births annually.

The first year of the study concerns the newborns of 1979. Approximately 600 of the transported infants are eligible for the study, and these will be matched by approximately 600 non-transported newborns. Data processing from hospital records (both sending and receiving), records of transport, and records of birth and death maintained by the NY City Health Department has begun. The data collection process is between 50-75% complete at this time, and preliminary analyses of the data are about to begin.

Sponsor: Department of Health, Education and Welfare, Public Health Service, Grant No. HPA 18HS03832

Principal Investigator: A.C. Ferrara, M.D., Ph.D.

Co-Investigator: M.S. Schwartz, M.D., Ph.D.

16. The Effect of Aircraft Noise on Children's Reading Ability in Brooklyn and Queens

The purpose of this study was to examine the relationship between aircraft noise levels and the percentage of students reading below grade level in all of the elementary schools in Brooklyn and Queens, NY. Multiple linear regression was used to control for confounding factors and to determine the significance of the noise exposure scores assigned to each school.

Brooklyn and Queens elementary school attendance zones were divided into five groups based on Noise Exposure Forecast (NEF) contours for John F. Kennedy (JFK) and LaGuardia airports. Schools in each noise group were assigned noise exposure scores roughly proportional to the noise energy. Because of the large reduction in the size of the noise-impacted areas around JFK during the 1970's, two noise scales were used. The Noise 70 scale was based on 1970 NEF contours for JFK and 1972 contours for LaGuardia. The Noise 78 scale was based on 1978 contours for JFK and the same 1972 contours for LaGuardia.

The validity of the Noise 78 scale was examined by correlation with noise level measurements made by the Federal Aviation Administration and

by the Environmental Protection Agency. The Noise 78 scale values for the measurement locations had a rank correlation of .0.95 with these measurements. The equivalent sound levels at these locations in Queens varied from 55 to 68 dB.

A magnetic tape obtained from the New York City Board of Education contained the aggregate results of the annual standardized tests of reading ability given in New York City Public schools and data on racial composition, socioeconomic level, and various educational factors for each school over the period 1972 to 1976. The percentage of students reading below grade level for each grade was regressed on the noise variable and the other variables for each of the years 1972-1976, and for all five years combined. Each observation was weighted proportionally to the number of students who took the reading test. Regressions using the combined five year data included indicator variables for the different years, to allow for temporal changes in reading level. Squared, square root, and interaction terms for the percentage of Black and Puerto Rican students, and for the percentage of those eligible for free lunch programs were included in the regression model, to remove abnormal trends in the plots of residual values against these variables.

Although the conclusions of this study must be qualified because of the less-than-ideal measures of noise exposure and socioeconomic status that were available and the aggregate nature of the statistics, the results support the hypothesis that high levels of environmental noise can affect reading ability. The estimated regression coefficients indicate that an additional 5% of the students in the noisiest shcools read at least one year below grade level, with 95% confidence limits from 2% to 8%. The dose-response relationship suggests that the effect of noise on reading ability increases with increasing noise level.

Sponsor: Institute of Environmental Medicine

Principal Investigator: B.S. Pasternack, Ph.D.

Co-Investigator: R.E. Shore, Ph.D.

Staff: K. Green, M.S.

Publication:

Green, K. *The Effects of Community Noise Exposure on the Reading and Hearing Ability of Brooklyn and Queens School Children.* Ph.D. Thesis, New York University, Graduate School of Arts and Science, October, 1980.

17. Mathematics of Carcinogenesis and Extrapolation to Small Risks for Carcinogens

The recent focus of this laboratory has been on the use of extrapolation to estimate population risks from low levels of human exposure to carcinogens, and on the prediction of the time pattern of induced cancer incidence after the introduction of carcinogens into the environment. Considerations of premature mortality introduce problems of competing risks and emphasize the importance of time-to-occurrence as a measure of carcinogenic response. Life shortening, as well as probability of occurrence, is an important index of the health impact of carcinogenic agents. Both parametric and nonparametric methods have been used to interpret animal and human data. In the context of low dose estimation of risks, the large uncertainties have motivated methods of Bayesian inference.

One of the recurrent issues in modeling carcinogenesis is the distinction between deterministic and stochastic aspects of the process. Emphasis on the deterministic aspects suggests that both time-to-occurrence and tolerance dose are log normally distributed. However, the stochastic approach is gradually receiving acceptance, and the multi-stage model is being adopted as an appropriately simple response model. This implies a polynomial form of cumulative hazard of which the Weibull distribution is a special case. The justification for the multi-stage model is a theoretical one which has been reviewed and extended by Whittemore and Keller in association with this Laboratory. However, the fact that it is difficult to obtain data to distinguish between the various models was illustrated by a detailed analysis of the human lung cancer incidence associated with cigarette smoking. Although the log normal distribution gave almost as good a fit, the Weibull distribution led to the simpler interpretation that incidence was proportional to dose and the fifth power of time.

The multi-stage model is also useful for predicting the time course of the rise of incidence in a population caused by the introduction of a new carcinogen into the environment such as the agricultural use of the organochlorine pesticides in the nineteen forties. A simulation study is under way, using equations derived from the multi-stage model, for a wide variety of special assumptions which can be reduced to a few canonical representations. Time to 50% maximum induced incidence varies from a few to more than fifty years, depending primarily upon the number of stages and the relative degree to which each stage is affected by the agent.

The problem of interpreting a no-observed-effect level has led this laboratory to become an advocate of Bayesian inference in special situations. When there are theoretical reasons for assuming a small but positive probability of response, the no-effect observation means that the smaller the estimate, the more likely it is, and the uncertainty in the estimate is best expressed in the form of a Bayesian probability

distribution based on a uniform prior. In the context of risk analysis, whenever the maximum likelihood estimate is much smaller than the upper confidence limit, practical interpretation should consider an analogous Bayesian approach. Bayesian probabilities as measures of degree of belief can be useful expressions of uncertainties which cannot be evaluated by objective statistical methods.

Dose-response models in carcinogenesis have been reviewed and a rationale has been given for fitting with a one-hit exponential response as an interpolative function for the low-dose data only. The higher dose groups with responses that do not fit with the one-hit model are dropped.

Sponsor: Institute of Environmental Medicine

Principal Investigator: B. Altshuler, Ph.D.

Staff: M. Gaffney, M.S.

Publications:

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18. Statistical and Epidemiological Methods

A recent paper (Haseman and Kupper, *Biometrics* 35:281, 1979) reviewed the various models, estimation procedures, and methods of analysis that have been proposed for the evaluation of binary responses that arise in toxicological experiments in which littermate data are frequently encountered. Haseman and Kupper also included suggestions for future research in this area pointing out, in particular, the need to reexamine existing data sets to assess the validity of various assumptions underlying models that are currently in use and to suggest alternative models that may be preferable, to devote more attention to the statistical aspects of the design of toxicological studies, to consider problems of multiple comparisons in the analysis of toxicological data, and to deal with the problem of establishing appropriate dose-response models for teratological-toxicological data and for extrapolating risk to low dose levels. During the past year, Pasternack and Shore have considered some new approaches to several of these issues, using data obtained from a toxicological investigation of the effects of DDT on stable laboratory mouse populations which was performed in the Institute's Laboratory of Toxicology.

It was found that both litter order and birth cohort can affect the 'litter lactation' index (d30/d4). These factors, along with litter size, were controlled for in Analysis of Variance (ANOVA) and Mantel-Haenszel (MH) analyses. ANOVA procedures based on transformed litter lactation indices (Freeman-Tukey binomial arc-sine [FTB] and the arc-sine [ARC]) yield conclusions similar to ANOVA procedures using the

untransformed lactation indices [RAW]. There is, however, a modest gain in power associated with the use of FTB and to a lesser extent ARC, compared to the use of RAW. It was also found that the use of the MH summary chi-square procedure based on a finite population approach, although somewhat less powerful than ANOVA, leads to similar findings concerning the effect of treatment when cohort, litter size, and litter order are controlled for by stratification. Again, the analyses based on FTB and ARC were superior to that based on RAW. If the assumptions underlying ANOVA are questionable in the analysis of dichotomous response data from toxicological experiments, the use of nonparametric methods (such as the MH procedure) are recommended.

Multiple tumors of a given type (e.g., skin or mammary tumors) are frequently encountered in studies involving human and animal carcinogenesis, yet satisfactory methods for analyzing such data have not been available. One approach uses the mean number of tumors per animal for group comparisons. However, when the survival experiences of the groups differ, this comparison is biased. Another approach is to use only the time to first tumor in life-table analyses. This method controls for variations in survival, but it ignores the information on multiple tumors.

Recently, Gail *et al.* (*Biometrics 36*:255, 1980) proposed methods for multiple-tumor analyses based on a semi-Markov approach in which the time to the kth tumor is conditioned on the time of occurrence of the (k-1)th tumor. This means that only the subjects who have the (k-1)th tumor are considered at risk for the kth tumor. Therefore, the risk sets become very small as k increases, and the comparisons are not very powerful, although they are more powerful than those of the single tumor analysis mentioned above.

A simpler and potentially more powerful 'nonparametric' method was investigated by Pasternack and Shore for the analysis of multiple tumors, which is applicable if the hazard-rate is comparable for the $1,\ldots,k$ tumors to be anlayzed. The method is based upon considering a subject to be at risk for tumor k as long as he is being observed and has had fewer than k tumors. A Mantel-Haenszel summary chi-square statistic is then computed over the risk sets defined by the k tumors. This approach is related, in a sense, to the concept of the "infinite-lived rat" considered by Altshuler (*Environ. Intnl. 1*:331, 1978) of this Institute's Biomathematics Laboratory.

To examine the assumption that the risk for developing a tumor depends on time, but not on the number of prior tumors, logrank analyses for the k risk sets, stratified by time and experimental group, can be performed. This assumption is currently being tested on several data sets, including data from an experiment by Morris and Burns on skin carcinogenesis in adult Swiss mice following *in utero* exposure to 7,12-dimethylbenzanthracene. If the assumption appears reasonable, the method will provide a powerful, unbiased, and relatively simple approach for analyzing multiple tumor data.

During the past year, work has been completed on the application of group sequential methods to the design and analysis of epidemiological studies. Computer simulation studies were performed which showed that group sequential methods can be employed when there are moderate variations in the size of the sequential groups, stratified data analyses are to be used, and the sample sizes for the two study groups are unequal.

A nonparametric life-table version of an earlier probabilistic model has been adapted by Dubin to assess lifetime benefits and risks of screening for disease. Discussions are currently underway with the HIP Breast Cancer Screening Project to obtain a data tape adequate for a full implementation of the nonparametric model. A flexible characterization is now possible for the radiation hazard, for other incidence and mortality functions, and for the number of screenings.

Sponsor: Institute of Environmental Medicine

Principal Investigator: B.S. Pasternack, Ph.D.

Co-Investigators: R.E. Shore, Ph.D., N. Dubin, Ph.D. and M. Marmor, Ph.D.

Publications:

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F. ENVIRONMENTAL POLLUTION AND ECOLOGY

1. Evaluation of Air Sampling Techniques Used to Measure Personal Exposures

Comparative sampling to determine the inhalation exposure of workers to airborne beryllium by personal sampling, and the time weighting of representative breathing zone samples result in significantly different exposure estimates (Donaldson and Stringer, *AIHAJ 41*:85, 1980). The exposure estimates obtained from full shift personal lapel monitors were two to three times higher than those determined by the method of time weighted averages (TWA). Sources of error which may contribute to this discrepancy are being investigated. The goal is to determine how to most closely approximate the true inhalation exposure.

To gather information on comparative sampling experience in other industries, a questionaire was distributed to about 100 members of the Lead Industries Association Environmental Health Committee, through the cooperation of Dr. Donald Lynam, Sr. of the International Lead Zinc Research Organization. Although 9 of the 13 respondents used both TWA and personal monitors, no comparative exposure estimates were available.

Sources of sampling error were separated for analysis into two classes, those which could result from characteristics of the sampling device, and those which might result from properties of the aerosol and its spatial distribution. Potential sources of error associated with sampling devices include flow rate calibrations, variable collection efficiency, self-dilution by either air cleaning or removal of contaminated air, inlet characteristics, and electric fields developed by polystyrene personal monitors. Aerosol distribution effects include fractionation of beryllium with particle size, the "pigpen" effect (i.e., that is, resuspension of dust from clothing, floors and other surfaces as a result of human activity) point sources which produce a non-uniform aerosol distribution, and electric fields developed by clothing which may perturb the aerosol distribution. Both laboratory and field studies are in progress to evaluate these possible sources of sampling error.

Laboratory studies have shown that the lack of agreement between the personal sampler measurements and the TWA values did not result from either flow rate discrepancies or problems related to filter efficiency. This was confirmed by laboratory analysis of a set of air samples taken at Brush Wellman, Inc. (BWI) by Dr. Mark Emly to test Whatman #41 and "filtrete" filter efficiencies.

Other laboratory studies which address sampling device error indicate that friction charging of personal monitors results in large electric fields (> 100 volts/cm) which persist for many days. Further work will quantitate the impact of such fields on aerosol sampling.

To assess the magnitude of the effect of sampling on an aerosol distribution or concentration, it is necessary to know what the true aerosol concentration would be in the absence of sampling. Two aerosol chambers have been constructed for these measurements, a glove box (volume = 0.88 m^3) and a larger "dust room" (volume = 5.3 m^3). It is difficult to uniformly disperse a sufficient quantity of dust for reliable measurements and to maintain a constant aerosol concentration. To circumvent these problems, a Wright Dust feed is used to rapidly generate a high dust concentration. Generation is stopped and the rate of aerosol decay is measured. For reasonably monodisperse particles with aerodynamic diameters of a few micrometers, a single exponential decay is observed. Thus, one can predict what the aerosol concentration would be in the absence of sampling and compare it with the measured concentration during and after sampling.

A series of experiments to evaluate self-dilution are in progress in the "dust room" utilizing magnetite particles (count median diameter = 1 μm and σ_g = 1.5). Preliminary results show that, for a well-mixed aerosol, a "Mini-hi volume" sampler of the type used at BWI for breathing zone samples (flow rate = 0.30 m^3 min^{-1}) will reduce the aerosol concentration at a rate which exceeds that expected. Other laboratory studies are underway to assess the aerosol concentration in the vicinity of the breathing zone.

Comparative measurements at the nose, lapel, and forehead have been made on personnel exposed to a submicrometer aerosol in a large chamber in which an elevated radon atmosphere is maintained. This chamber is maintained at the Environmental Measurements Laboratory of the DOE. Radioactive short-lived radon daughters provide a convenient tag for the particles collected on air samples. In the test chamber, as in most environments, the AMAD of the aerosol to which radon daughters are attached is about 0.12 μm. It was found that, for submicrometer particles, there is no difference in performance between samplers placed at the lapel and at the nose. Similar measurements are being made for larger particles dispersed with the Wright Dust feed into the dust room. For these studies, samplers are placed at the positions of interest on a mannekin. Exposures will be measured by the different samplers for both well-mixed and non-uniform dust distributions.

Field experiments have been carried out at the beryllium production facility in Elmore, OH. Preliminary nephelometric measurements with a real-time aerosol monitor (RAM) have characterized the temporal variations in the breathing zone of a worker during a beryllium alloy metal casting cycle. A portable device incorporating 4 RAMs is being developed for more detailed field monitoring.

The use of multicyclone samplers to characterize particle size distribution was tested for three industrial process areas. The particle size distribution of the aerosol at the production facility is not

known. In general, particle sizes are related to the process by which they are generated. Particle size distribution may thus be expected to vary if local sources predominate in different plant areas. In addition, the specific concentration (μg Be/μg of aerosol) of the Be in the workplace aerosol may differ for each process area.

A simplified direct current plasma emission spectrometric method with almost no interferences has been developed to measure trace amounts of beryllium in aerosol samples and in some organic matrices. Analysis of blanks and spiked samples were performed as quality control measures. Also, field samples collected on different filter media were anlayzed. The working linear range is between 0.002 ppm and 5 ppm. This technique was developed in cooperation with the Environmental Measurements Laboratory of the Department of Energy, where all of the measurements were performed.

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2. Aerosol Composition and Health Effects Studies

Increased use of coal for power production and a shift to diesel-powered automobiles have been proposed to mitigate energy resource problems in the United States. Studies in this laboratory have shown that changes in fuel use patterns can have substantial impact on the urban aerosol. These studies have traced declines in total suspended particulate matter (TSP) in N.Y.C., due to emission regulations, from 135 $\mu g/m^3$ in 1969 to 54 $\mu g/m^3$ in 1978. Through the application of a multiple regression model developed for the purpose, it has been possible to estimate the contributions of the major sources of pollution to ambient TSP levels. This model is of the form TSP = 12.0 (Pb) + 54 (Cu) + 103 (V) + 1.66 (SO_4) + 420 (Mn) + 26.8, for the years 1972-1976. In this model, TSP and the tracers are expressed in $\mu g/m^3$. Each of these trace elements has been shown to have one predominant source in New York City, lead from autos, vanadium from oil burning sources, copper from incinerators, manganese from soil, and sulfate from secondary formation processes.

a. Trends in Particulate Pollution

Improvements in air quality were previously reported for total suspended particulate matter and for various trace element concentra-

tions between 1972 and 1975. Lead continued to decrease from 1975-1978 because of the increasing use of no-lead gasoline. A strong seasonal influence has always been present for vanadium because of the heavy use of oil for heating in the winter. Since 1978 a shift toward higher values has been noted for winter quarter TSP, respirable suspended particulate matter (RSP) and vanadium. These upward shifts have occurred despite warmer winters in 1978-1979 and 1979-1980, compared to 1977-1978 and earlier years.

There is no indication of a shift in SO_2 or $SO_4^=$ levels, and benzo-(a)pyrene (BaP) concentrations correlate with degree days. It appears that both fuel use, as indicated by BaP, and sulfur emissions have remained in control.

The deterioration of air quality since 1978 was not predicted, and it cannot be explained by meteorological changes. Evidence is being sought to determine the effect of shifts in oil production sources subsequent to the summer of 1978. It is expected that any major change in the country of origin of low sulfur crude oil or crude oil desulfurized to produce No. 4 or No. 6 oils could explain a significant shift in emissions.

Shifts in emission rates or in the composition of emitted particles require changes in the regression models or chemical element balance models, as the coefficients in such models must be representative of the emissions being characterized. These mathematical source apportionment techniques are beginning to be used to guide regulatory decisions. It is important to recognize the potential impact of fuel quality shifts on such models.

b. Trace Organic Compounds: Source Contributions to the New York City Atmosphere

Particulate organic matter (POM) is a significant fraction of the aerosol mass. (up to 25%). The nonpolar fraction which is extractable with cyclohexane or benzene is comprised of many compounds known to be carcinogens, co-carcinogens, mutagens, irritants, etc. Investigations in this laboratory over the past few years have also indicated the presence of biologically active compounds in the polar fractions of POM including the N-nitrosamines, alkylating agents and other compounds which are direct-acting mutagens in the Ames bioassay. Since these materials may pose a health hazard, it is important to develop methods to identify their major sources.

As part of the research program on organic compounds in the atmosphere, source apportionment models similar to those developed for TSP have been developed for POM fractions and compounds in the atmosphere. Using these models, it has been possible for the first time to obtain estimates of the contributions of various types of sources to airborne concentrations of POM.

The data base used for the development of this model included masses of three POM fractions extracted sequentially with the increasingly polar solvents, cyclohexane (CYC), dichloromethane (DCM) and acetone (ACE). The other variables included were benzo(a)pyrene (BAP) and chrysene (CHRY), trace metals (Pb, Cu, V, Mn), sulfate and meteorological parameters. Principal factoring (R-analysis) with a varimax rotation was performed on these data and on various subsets. Depending upon the number of variables included in the analysis, three to five factors were obtained which could be related to emission sources through trace metals associated with specific types of sources. A meteorological factor, which includes the variables wind speed and mixing height, has been found to be very significant, confirming previous work in this laboratory by Kleinman and co-workers.

Based on the selection of source tracers from the factor analysis, linear multiple regression models were developed for two organic fractions (CYC and ACE), which together constitute 90% of the POM in New York City. Preliminary models have also been developed for two polycyclic aromatic hydrocarbons, BAP and CHRY, as well.

As an example, the model for cyclohexane-soluble (non-polar) organics is CYC ($\mu g/m^3$) = 29 ± 4 [V] + 0.65 ± 0.35 [Pb] + 1.0. Using the average ambient concentrations of Pb and V as tracers for oil-burning and automotive emissions, these sources were estimated to contribute 1.7 and 0.6 $\mu g/m^3$, respectively. The models for the CYC and ACE fractions together indicate that 40% (3.0 $\mu g/m^3$) of the solvent-extractable organics in respirable particles in New York City were associated with oil-burning, 19% (1.4 $\mu g/m^3$) was from automotive sources and 15% (1.1 $\mu g/m^3$) was associated with soil-like particles. The preliminary models for benzo-(a)pyrene indicate that 70% of this compound orginated from oil-burning sources in the winter months.

As a test of the models, the coefficients obtained from the multiple regression analysis were compared to available source emission data. In general, the available source emission data support the validity of the models.

Efforts to develop similar source apportionment models for N-nitrosamines, alkylating agents and mutagenic compounds in airborne particulate matter are in progress. The strong seasonal patterns in concentration which have been observed (i.e., winter maxima and summer minima) suggest that oil-burning for winter space heating is a major source of all of these materials.

c. N-nitrosamines in Airborne Particulate Matter

Research in other laboratories on N-nitrosamines in the ambient atmosphere has been directed toward analysis of volatile or gas phase species. During 1978-79, an analytical method which is specific for the N-nitroso group was developed in this laboratory for use on extracts of particulate matter samples.

Using this method, evidence of the presence of previously undetected and unidentified non-volatile, N-nitrosamines in ambient particulate matter was found. Over the past year, efforts have focused on confirmation of the presence of the N-nitroso functional group and identification of specific compounds.

The N-nitrosamine-containing fraction isolated from ambient particulate matter collected in New York City was separated by both thin-layer and high-pressure liquid chromatography, to isolate N-nitrosamines from other materials for mass spectrometric identification. Two class-specific assays have confirmed the presence of these compounds in the fractions which have been isolated. The retention times of the still unidentified compounds in these fractions do not correspond to those of available N-nitrosamine standards, such as dimethylnitrosamine, diethylnitrosamine, dibutylnitrosamine, N-nitrosopiperidine and N-nitrosopyrrolidine. Instead, they appear to be representative of nitrosamine compounds of higher molecular weight.

Weekly samples of respirable suspended particulates (RSP $\leq$ 3.5 µm mmad) collected from January, 1978 to September, 1979 in New York City have been analyzed for the presence of N-nitrosamines, using the previously developed assay. During this period, the mean concentration of particulate N-nitrosamine was 78 picomoles/m^3, with a range from 0 to 335 picomoles/m^3. This is in the same range as the concentrations which have been observed for vapor phase nitrosamines in urban areas.

A seasonal pattern, with elevated concentrations in the winter similar to that observed for other organic fractions and for bacterial mutagens, was demonstrated by these measurements. This indicates that oil-burning for space heating is a source of these compounds. Statistical correlations obtained by mathematical analysis between the concentrations of N-nitrosamines and V, Pb and Mn, tracers for fuel oil burning, automobiles and soil-like particles, respectively, indicate that other sources may contribute to the N-nitrosamine concentration as well.

Further work has been done comparing the literature data on the carcinogenicity of N-nitrosamines to that for benzo(a)pyrene (BaP), to estimate relative risks. Collected and pre-sorted BaP and N-nitrosamine data have undergone further sorting to remove all cases having very small groups of animals or complicating secondary treatments. Attempts to find a best-fit, non-complex curve for the compiled BaP carcinogenicity data have led to a response versus log-log dose relationship. Fitting the N-nitrosamine data to a similar curve is continuing. Once the fitting is completed, direct comparison of relative dose effects of the two compound classes will be possible at extrapolated levels comparable to ambient levels.

d. Mutagenic Activity of Airborne Particulate Organic Matter

Seasonal variations in bacterial mutagenic activity have been found in three sequentially extracted organic fractions of respirable particu-

late matter (RSP) in aerosol samples collected on fiberglass filters in New York City from July, 1977 to March, 1979. In general, the cyclohexane, dichloromethane and acetone fractions, containing non-polar, moderately-polar, and polar compounds, respectively, showed clear fall-winter maxima and spring-summer minima in revertants produced by direct-acting mutagens with both Salmonella strains TA-98 and TA-100. The activity per cubic meter of air was approximately twice as high for the fall/winter period as for the summer period for both the non-polar and polar fractions (significant at $p \geq 0.01$), while the seasonal differences were not as great for the moderately polar fraction.

Two factors contribute to the fall/winter maxima in mutagenic activity per cubic meter observed. First, the atmospheric concentrations of the particulate organic matter tend to reach a maximum during the fall and winter seasons, and a minimum during the summer, particularly for the non-polar and polar fractions. Secondly, the net activity per microgram for these two fractions is also slightly higher during winter than summer.

Since preliminary investigations indicated that the moderately polar dichloromethane extracts were the most active per unit mass, fractionation and identification work has concentrated on this fraction. Initial fractionation of a yearly composite of 52 weekly dichloromethane extracts on a Perkin-Elmer reverse phase HPLC column, using a linear solvent program of 100% water to 100% methanol, yielded a chromatogram with more than 40 distinct peaks. Mutagenic activity was found in fractions of intermediate polarity. For example, one such fraction showed 15 revertants per microgram of material, while the original composite yielded 8.6 revertants per microgram.

Since differences in mutagenic activity have been observed in the quarterly composites, HPLC profiles of the remaining samples of each of the seasonal composites are now being taken to determine whether qualitative and/or quantitative differences in the chromatographic peaks can be observed. Appropriate fractions will be isolated and tested for mutagenic activity, in preparation for further chemical classification of the biologically active material.

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3. Organic Materials in Urban Atmospheres: Degradation and Biological Responses

a. Sampling Artifacts in Measurement of Organic Constituents of Urban Aerosols

Determination of atmospheric concentrations of particulate organic matter (POM) in both urban and industrial environments generally involves collection of particulate matter on a filter and then extraction of the organic fraction with organic solvent(s). The amount of extractable material and, in many instances, the concentrations of specific organic compounds are then determined to describe potential occupational or community exposures.

A review of the few available studies on sampling artifacts associated with the collection of particulate organics on glass fiber filters suggests the possibility of losses in hydrocarbon content when samples are collected for the conventional twenty-four hour sampling period. Thus, reported concentrations of non-polar hydrocarbons in ambient and industrial atmospheres may be underestimated. In view of this possibility, an investigation of artifacts in sampling for particulate organic compounds was initiated. The first step in this investigation was to determine whether any relationship exists between the period of sampling and the apparent atmospheric concentration of POM collected on glass fiber filters.

Samples of total suspended particulate matter were simultaneously collected over three sampling periods: seven days (1 weekly sample), twenty-four hours (13 daily samples), and six hours (4 samples/day for 13 days). Each collected filter sample was extracted sequentially with solvents of increasing polarity, cyclohexane, dichloromethane, and acetone, to separate non-polar, moderately polar and polar organics, respectively.

The apparent atmospheric concentrations of cyclohexane-soluble POM were 35% higher on average for 24-hr samples than for 6-hr samples. The weekly sample, in turn, showed a similar increase in apparent concentration, relative to the 24-hr samples for this fraction.

Gas chromatographic analysis of composited 6-and 24-hr samples, and of a weekly sample of the cyclohexane-soluble POM, showed that losses of aliphatic hydrocarbons below the molecular weight of n-nonadecane were associated with the longer sampling times, compared to the 6-hr samples. However, there was also an increase in the concentration of higher molecular weight hydrocarbons with longer sampling periods. This increase more than compensated for volatilization losses.

In contrast to the cyclohexane-soluble POM, the dichloromethane-soluble POM exhibited lower apparent concentrations with longer sampling times. It is not clear at present whether this is due to volatilization or oxidation losses, or possibly both. No statistically significant difference in apparent concentration with increased sampling period was observed for the acetone-soluble fraction of POM. No obvious relationship between the shifts in the observed values for the non-polar and moderately polar fraction or the absence of change in polar materials was found.

These results clearly demonstrate that sampling period duration is an important factor in determining the apparent atmospheric concentrations of some POM fractions. The observations made for the cyclohexane-soluble POM show that sampling mixtures of airborne organics is quite complex, and both negative and positive artifacts may contribute to the apparent concentration. Further experiments are in progress to determine the effect of sampling period on the mutagenic activity of POM in the Ames assay.

b. Cell Transformation

Cell culture systems have been used increasingly in cancer research to study the effects of environmental agents which may cause or contribute to cancer. Such *in vitro* systems have both advantages and disadvantages compared to *in vivo* methods.

A number of agents have been found to be carcinogenic in mouse skin only when applied together with subeffective doses of a known carcinogen. Most demonstrations of this process involve *in vivo* systems that do not lend themselves to critical analysis at the cellular and biochemical level. It is the intent of this study to develop an *in vitro* assay for the identification of cocarcinogenic compounds, as well as to demonstrate the process of cocarcinogenesis in both human and rodent cell cultures.

Cocarcinogenic compounds, catechol, pyrene and fluoranthene, are being administrated to Balb/3T3 and WI-38 cell cultures, together with subeffective doses of BaP, to develop a cell transformation system which shows a response similar to that of mouse skin. Dose-response data have

been obtained for the toxic effects of BaP and catechol, to establish acceptable concentration ranges for transformation studies. A protocol has been followed in testing for transformation by a cocarcinogenic effect which includes both positive (BaP only) and negative controls, along with a subeffective BaP treatment with varying concentrations of catechol. Transformation has been achieved, which has been detected by morphological and colony growth characteristics, including the ability of the transformed cells to grow in soft agar. The transformed cell lines are now being characterized.

The protocol developed appears to be effective, and it may afford a relatively rapid screening method for cocarcinogens. The system should also provide a basis for the examination of biochemical or other cellular changes which occur during transformation.

c. Transport of Polycyclic Aromatic Hydrocarbons (PAH) into New York City

Summertime transport of sulfur dioxide and particulate sulfate from oil and coal-burning areas in the midwest to the northeastern part of the U.S. has been well documented. Organic compounds have not been as extensively investigated in this respect, although there have been several reports of the transport of polycyclic aromatic hydrocarbons (PAH) produced by fossil fuel combustion to rural and remote areas.

During the past year, the variations in ambient concentrations of PAH compounds found during the initial New York Summer Aerosol Study in 1976 were examined, in conjunction with meteorological data. These data suggest that long-range transport of atmospheric aerosol can contribute to PAH concentrations in New York City.

The PAH measurements made during two weeks in the summer of 1976 indicate three different patterns for the variations in atmospheric concentrations of these materials, along with the more commonly measured constituents of particulate sulfate and ozone:

Type I - transport of recent emissions of PAH in which high concentrations were associated with the initial phases of transport of high pressure systems from the midwest, with high sulfate concentrations and high benzo(a)pyrene (BaP)/benzo(ghi)perylene (BghiP) ratios;

Type II - transport and degradation of PAH in which low concentrations were associated with the return flow of an aged high pressure system that had persisted over the eastern U.S. and had accumulated high levels of sulfate aerosol;

Type III- insignificant transport of PAH in which low concentrations were associated with airflow from clean air regions.

When compared to wintertime values, the levels encountered during an episode of transport of recent Type I emissions were not excessive. However, the results warrant further study, in light of current re-emphasis on coal burning for energy production. The observations of increased ratios for BaP/BghiP during transport episodes suggest that this ratio may prove useful in determining the origin and/or age of air masses affecting rural or urban locations.

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4. Photodegradation of Adsorbed Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAH) in the atmosphere can be associated with particulate substrates which differ considerably depending upon their source. These differences in substrate composition can have a substantial impact upon the PAH half lives, reaction products and biological activity. Since there is little or no information on the half-lives of adsorbed PAH, an investigation has been initiated on the photodegradation of these compounds under simulated environmental conditions. The objectives of this study are:

1. to design, construct and evaluate a fluidized-bed photochemical reactor for laboratory studies of photodegradation of adsorbed PAH;

2. to determine the rates and products of photodegradation of adsorbed PAH compounds; and,

3. to determine the stability of some PAH epoxides (possible intermediates in photodegradation) adsorbed onto various substrates.

The photoreactor designed and constructed for this investigation is a glass, water-jacketed column, 250 cm in length, and 2.4 cm in diameter, with fritted discs at the base and top of the column which allow a flow of gas to move the particles. A particle mass load of 0.5 to 1.0 g and gas flows of 10-20 l/min give a bed height of ~10 cm, the length of a 200 watt quartz mercury-vapor lamp used for irradiation. The glass reactor acts as a filter for ultraviolet wavelengths, and thus, there is a light spectrum in the reactor similar to that of sunlight.

Methods have been developed for the reproducible adsorption and extraction of pyrene on glass beads and on Carbosieve S, a carbon substrate. Extraction efficiencies of 90-100% have been found for Carbosieve S with CS_2 and for glass beads with methanol. High-pressure liquid chromatographic (HPLC) methods have been developed for the separation and analysis of pyrene and the oxidized derivatives of pyrene produced by degradation reactions.

The rate of degradation of pyrene under various conditions has been investigated. Degradation appears to follow a first-order rate law and to be dependent upon the presence of oxygen. Analysis of the air within the reactor showed that the levels of ozone were less than 10 ppb, the detection limit of the analytical method. Irradiation was not required for the degradation of pyrene adsorbed on glass beads.

The average rate of degradation for pyrene on 75-150 μm diameter glass beads was found to be $5.1 \pm 0.8 \times 10^{-3}$ min^{-1} ($t_{\frac{1}{2}}$ = 135 min) for a coating of more than 0.3 mg pyrene/g glass, and $12 \pm 2 \times 10^{-3}$ min^{-1} ($t_{\frac{1}{2}}$ = 58 min) for a monolayer coating of 0.07 mg/g. The rates are quite reproducible. Using HPLC and appropriate standards, 1,6-, 1,8- and 4,5-pyrene dione, a pyrene diol and 1,1-bipyrene were identified as products produced in the degradation of pyrene on glass beads.

In preliminary experiments on the degradation of pyrene absorbed on coal fly ash, a rate constant of 3×10^{-3} min^{-1} ($t_{\frac{1}{2}}$ = 230 min) was found. Thus, pyrene appears to be more stable on coal fly ash, a material found in the environment, than on glass.

In addition to the continuing experiments to determine the rates and products of degradation of a number of polycyclic aromatic hydrocarbons on various substrates, this work is being extended to include the incorporation of a chemical actinometer, to determine light intensity in the reactor; to the investigation of the biological activity of the products of degradation, using the Ames bioassay; and to the elucidation of reaction mechanisms.

The information obtained from this investigation will be useful for evaluating the potential health hazards associated with particulate PAH in atmospheric aerosols, from increasing coal utilization in the U.S.

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5. Strong Acid Aerosol

During 1976 and 1977, ambient strong acid (H^+) was measured at rural sites around the N.Y. metropolitan area and at the Medical Center in mid-Manhattan. The highest concentrations were measured at High Point, NJ, which is located west-northwest of New York City, and at Mid-Manhattan, with the highest 6-hr. sample concentrations being 25 to 35 $\mu g/m^3$. Other studies conducted in the eastern U.S. also have shown the accumulation of acid aerosol during the summer. Health effects studies conducted in this Institute with H_2SO_4 concentrations of 100 $\mu g/m^3$ have shown an effect on the mucociliary clearance of nonsmoking healthy adults who were exposed for periods of 1 hour. The present study was initiated to investigate the frequency and the intensity of occurrence of ambient acid aerosol during persistent elevated pollution episodes in the summertime, and to determine if there is a potential for health stress in rural environments.

The study is being conducted at a site in Sterling Forest, N.Y. near the Lanza Laboratory. The location is approximately 70 km north-northwest of New York City, and because of prevailing wind patterns, it is usually not affected by local pollution in the summertime.

Several analytical techniques are being used for this investigation. The first involves sampling for 12 hr. on a H_3PO_4-pretreated quartz filter. These samples are analyzed for strong acid by calorimetric titration and for SO_4 by turbidimetric analysis, after precipitation with barium. Analysis of the H_2SO_4 and its ammonium salts is done *in situ* with a continuous flame photometric sulfur detector.

A sampling study was conducted during the period from July 30, 1980 through August 29, 1980. Intense heat waves occurred in the eastern U.S. during the first and last weeks of the month, with temperatures in excess of 90°F. The filter samples have been extracted and are now being analyzed. The particulate mass results show peak 12 hr. values of 111 and 100 $\mu g/m^3$ for August 4 and 27, respectively. The minimum mass values recorded were < 20 $\mu g/m^3$. The *in situ* sulfur analyzer was operated from August 15, through September 1, 1980, and preliminary results indicate the that the H_2SO_4 concentration was > 4 $\mu g/m^3$ on August 27.

Sponsor: The New York State Health Research Council, Grant No. HRC-1755

Principal Invstigator: P.J. Lioy, Ph.D.

Co-Investigators: T.J. Kneip, Ph.D. and M. Lippmann, Ph.D.

Staff: M. Morandi, B.S.

6. Seasonal Sulfur Dioxide and Sulfate Variations

The distribution and sources of the sulfate aerosol in New York City continue to be of concern because of their preponderance in the respirable particulate fraction and the anticipated return to the combustion of coal and high sulfur oil for the production of heat and electricity. A study is being conducted which involves the comparison of the summer and winter concentrations of SO_2 and SO_4 observed in NYC, from 1972 through 1979.

The concentrations of sulfur oxides in New York City declined between the years 1964-1972. The primary reasons for this decrease were the phasing out of coal combustion for heating and generating electricity, and the regulation of the sulfur content of fuel oil. Since 1972, fairly low SO_2 concentrations have been observed because of the adoption of regulations limiting the sulfur content in fuel oil to < 0.3%. Significant increases in the seasonal average concentrations of vanadium (V), sulfur dioxide (SO_2), and total suspended particulates (TSP) from 1972 to the present have been observed, primarily at times when 0.3% S oil was in short supply and had to be supplemented with higher sulfur fuels. Such periods were the winters of 1973-74 and 1976-77.

The data used in this study were obtained from the New York University Medical Center rooftop station, the Roosevelt Island, State Monitoring site, and the City College of New York State Monitoring site. Supplemental data were retrieved from the other state SO_2 monitoring sites.

The arithmetic mean $SO_4^=$, SO_2 and V concentrations were calculated for the winter and summer at three different sites for the period 1972-1979, and these were compared with an SO_2 emission inventory for the years 1972-77. It was found that summer ambient $SO_4^=$ levels were as high as or higher than winter $SO_4^=$ concentrations, while SO_2 and V ambient levels were higher during the winter. The latter levels parallel the peak seasonal use of fuel oil in NYC, with periods of high sulfur fuel use having notably higher values. When the SO_2, $SO_4^=$ and vanadium concentrations were normalized for atmospheric dispersion, only the $SO_4^=$ values showed a changed pattern; normalized $SO_4^=$ became higher in winter. Normalized winter/summer ratios for V and SO_2 increased over those for uncorrected data. This suggests that during the winter, sulfate was primarily from local sources, but the actual concentrations were reduced because of enhanced dispersion. The daily data from the winter studies showed a high correlation between SO_2 and $SO_4^=$ levels, indicating that local SO_2 emissions and transformation, either at the source or in the ambient air, accounted for much of the winter $SO_4^=$.

The 1976 and 1977 summer results showed a low correlation between SO_2 and $SO_4^=$. This result, along with detailed trajectory studies, indicates that regional SO_2 emissions and transformation processes are responsible for the high summer sulfate levels. These analyses help to explain the occurrence of the high ambient $SO_4^=$ levels in NY City during the summer since 1972, despite the low summer SO_2 levels observed.

Sponsor: Institute of Environmental Medicine

Principal Investigator: P.J. Lioy, Ph.D.

Co-Investigator: T.J. Kneip, Ph.D.

Staff: M. Morandi, B.S., P. Mallon, M.S. and J. Miller

Publication:

Lioy, P.J. and M. Morandi. The seasonal levels and origin of SO_2 and $SO_4^=$ in New York City. In: *Proceedings of the 73rd Annual APCA Meeting*, #80-51.3, Montreal, Quebec, June, 1980.

7. Long-Range Transport of Particulate Organic Pollutants

Recent studies suggest that particulate polycyclic aromatic hydrocarbons (PAH) produced by combustion processes can be transported over long distances. Since increased utilization of coal is planned in the U.S., and combustion of coal generates higher levels of PAH compounds than oil, it is important to determine the lifetimes of such compounds in the environment.

The Arctic is an ideal area to investigate the possible transport of particulate air pollutants, as there are few local sources of airborne pollutants. Recent investigations of the Arctic aerosol have revealed the presence of relatively high concentrations of sulfate during the winter months, derived largely through conversion of SO_2 to sulfate during the transport of polluted air masses from mid-latitude regions. During these investigations, it was observed that samples of particulate matter were generally grey when collected in winter and pale yellow in summer. This suggested the presence of elemental carbon and associated particulate organic matter (POM), such as the polycyclic aromatic hydrocarbons. In a cooperative study with Dr. Kenneth Rahn of the University of Rhode Island, samples of total suspended particulate matter were collected in March and August, 1979 at Barrow, Alaska, a remote site in the Arctic.

The samples were analyzed for soluble POM, for PAH compounds and for ^{210}Pb, and they were also examined by optical and scanning electron microscopy. The concentrations of solvent-extractable POM were similar to those reported for other remote sites in the northern hemisphere (0.2-1.5 $\mu g/m^3$), but there was a strong March maximum, similar to that observed for sulfate aerosol. Average concentrations of the sum of eleven PAH compounds were 1.2 ng/m^3 and 0.15 ng/m^3 for March and August, respectively. Microscopic examination revealed the presence of fly ash in March, while pollen grains predominated in the August samples. Levels of ^{210}Pb associated with the airborne particles were an order of magnitude higher in March than in August, and they were also higher than those observed in mid-latitude urban areas. This indicates that an aged aerosol was sampled during March. Seasonal differences in meteorology and in airborne concentrations of organic species and ^{210}Pb, as well as the presence of fly ash in the samples collected in March, 1979 indicate that the principal source of particulate organic species during this period was fossil fuel combustion in the mid-latitudes. This implies long-range transport of these species and lifetimes of the order of weeks, rather than days.

It has often been assumed that pollution-derived particulate organic species, PAHs in particular, are relatively short-lived in the atmosphere. This research has shown that pollution-derived organic species can impact on regions far removed from their sources under certain conditions, and that control of air pollution will require regional and international cooperation.

Sponsor: National Institute of Environmental Health Sciences, Grant No. ES 01691

Samples were collected as part of the cooperative program with the National Oceanic and Atmospheric Administration/Geophysical Monitoring for Climatic Change Program.

Principal Investigator: J.M. Daisey, Ph.D.

Publication:

Daisey, J.M., R.J. McCaffrey and R.A. Gallagher. Polycyclic aromatic hydrocarbons and total extractable particulate organic matter in the arctic aerosol. *Atmos. Environ.* In Press.

8. The Relationship Between Haze, Ozone, Sulfate and Transport (HOST)

A number of studies have indicated an association of ozone and sulfate with long-range transport processes within high pressure systems, when elevated concentrations of ozone (> 120 ppb) and sulfate (> 12 $\mu g/m^3$) were widespread in the summer. In most of these studies, however, accumulation and transport patterns for ozone and sulfate have been examined independently.

The present analyses attempt to establish, through statistical methods, relationships among the O_3, $SO_4^=$ and visibility data observed at various locations in the Northeast Quadrant of the U.S. Two types of data sets are being analyzed, one including all available coincident data, and one limited to data for episodic meteorological situations.

The data used for this study were acquired from a number of sources. The ozone concentrations were collected from the monitoring networks of 19 states within the Northeast Quadrant of the U.S., and the values used were the daily one-hour maximum concentrations. The daily maximum value has been commonly regarded as representing the ozone concentration available in the mixed layer of an air mass, since local sources have been shown to produce a negative interference, reducing the ozone concentration at times of the day other than that for the maximum value. The periods included in the data base were April 1 through August 31, 1976; August 1 through 31, 1977; and June 1 through 30, 1978.

The data for the entire 1976 photochemical oxidant season were included to provide a calibration year for selection of "subregional" ozone monitoring sites. In the present instance "subregional" is defined as specific sites that are highly correlated with other sites within a 80 to 120 km radius, for various locations in the Northeast Quadrant of the U.S.

The sulfate values (24 hr samples) used in these analyses came from a number of sources. A major portion of the data was obtained from the SURE monitoring network which operated in August, 1977 and July, 1978. In addition, data for the New York Summer Aerosol Study were obtained for the periods of July and August, 1976, and August, 1977. All sulfate values reported herein were 24 hr samples (except for New York Summer Aerosol Study, August, 1977, which were volume-weighted averages of four 6-hr samples) and were the water-soluble sulfates associated with each sample.

The statistical analyses indicate that there is a significant correlation (by the method of Spearman, Kendall and Pearson) between sulfate and ozone, with the highest coefficients ($r > 0.5$) usually occurring for same day analyses and for ozone sites within 100 km of a particular $SO_4^=$ site.

Isopleths of the statistical significance of the ozone correlations with sulfate have been generated for each site with valid sulfate data. Three patterns of association were observed:

1. A pattern of a well-defined west to east gradient of increasing significance of correlation of ozone values (for 2-days previous, 1-day previous and same-day) to $SO_4^=$ values, with the same-day isopleths enveloping areas close to and around the $SO_4^=$ receptor site.

2. A large overlap of all three significance isopleths (2-days previous, 1-day previous and same-day ozone) associated with an $SO_4^=$ receptor site.

3. The absence of a defined relationship between significance isopleths other than between the same-day ozone and $SO_4^=$ values for a given $SO_4^=$ receptor site.

In the above analyses all available $SO_4^=$ sampling days were used. Similar results were also observed when the data base was truncated to the periods of pollution episodes.

Presently, trajectory analyses have been completed for the sites of Lewisburgh, WV; Giles City, TN; and Duncan Falls, OH for August, 1977. The results support the findings of the statistical studies. These trajectories include both a 36-48 hr backward component (with the site as receptor), and a 24-48 hr forward component (with the site as a source region). The results indicate that Lewisburgh, WV was usually affected by air which had passed over the major source regions in the eastern half of the U.S. (e.g., the Tennessee Valley and industrial mid-west), and that once the air passed through the Lewisburgh area it was frequently transported to the northeast. For Giles City, TN, the air usually had been over the southeastern U.S. before passing over the site, but it was occasionally affected by local stagnation. As in the

Lewisburgh, WV case, however, the downwind areas were usually toward the northeast. The Duncan Falls trajectories showed a pattern similar to that observed at Lewisburgh, with the dominant forward direction being toward the northeast.

The results to date clearly support the hypothesis that the sulfate and ozone levels observed in separate studies are strongly interrelated.

Sponsor: U.S. Environmental Protection Agency, Grant No. R806593-01-1

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9. Fallout $^{239,240}Pu$ in Human Tissues

Since World War II, the world's population has been exposed to isotopes of plutonium due to the dispersion of approximately 350 kCi of $^{239,240}Pu$ from atmospheric nuclear weapons testing. Various studies were begun where samples of air, precipitation, food, and biological tissues were collected and analyzed to document the transfer of these isotopes from the environment to man. The results of these and other investigations indicated that the gastrointenstinal tract uptake factor for fallout plutonium was extremely small (approximately 10^{-6}), and that inhalation was the predominant chronic exposure route.

The purpose of this study has been to directly determine the concentration and distribution of plutonium in the tissues of non-occupationally exposed individuals. Data obtained could then be used to construct metabolic models for the evaluation of possible future exposures to environmentally-dispersed plutonium.

Analyses were conducted on tissues obtained from four geographical locations including New York City, Washington, DC, Mobile, AL and Grand Junction, CO. Tissues were acquired at autopsy from accident victims. When possible, each tissue set consisted of the right lung, peritracheal, peribronchial and hylar lymph nodes, 500 g of liver, one kidney, and half of the bodies of three thoracic vertebrae. Epidemiological information was also obtained to investigate any possible correlations between this information and plutonium concentration.

Initial studies were conducted on autopsy tissue samples obtained from residents of New York City. Subjects within the study group ranged in age from 15 to 82 years, with more than half being between 20 and 40 years old. A total of 57 cases were analyzed, and median $^{239,240}Pu$ values of 0.37 pCi/kg for liver, 0.25 pCi/kg for vertebrae, 0.11 pCi/kg for T-B lymph nodes, 0.055 pCi/kg for lung, and 0.050 pCi/kg for kidney were obtained.

More recently, tissue samples were obtained from residents of Washington, DC and Grand Junction, CO whose median ages were 33 and 67 years, respectively. Ten sample sets from Washington gave median $^{239,240}Pu$ values of 0.60 pCi/kg for liver, 0.17 pCi/kg for vertebrae, 0.46 pCi/kg for lymph nodes, 0.08 pCi/kg for lung and 0.02 pCi/kg for kidney. Ten samples were also analyzed from Grand Junction which gave median values of 0.55, 0.22, 0.68, 0.07, and 0.03 pCi/kg for liver, vertebrae, lymph nodes, lung, and kidney, respectively.

Plutonium concentrations for each tissue were characterized by a log-normal distribution. No significant statistical differences in median plutonium concentrations other than in lymph nodes were detected among the three populations. The difference in lymph node values has been attributed to more careful acquisition and larger sample sizes of lymph nodes from Washington and Grand Junction, which led to lower

sample losses and statistical errors. When tested by non-parametric methods, no significant correlations were found between $^{239,240}Pu$ concentrations and any of the epidemiological factors of age, sex, smoking history, weight, and stable element concentration.

Additional tissue samples have now been acquired which are undergoing chemical analysis. These samples include 14 more tissue sets from Washington, DC and 8 bone sets from Mobile, AL. The bone sets include various bone types including tibia, femur, ribs, and vertebrae from adults and from three fetuses. Initial analyses of plutonium concentrations in these bones have provided valuable data for describing the distribution of this nuclide at various trabecular and cortical sites in the skeleton.

Sponsor: U.S. Department of Energy, Contract No. DE-AS02-76EV-02968

Principal Investigator: N. Cohen, Ph.D.

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10. Abundance and Distribution of PCBs in Hudson River Plankton

Environmental monitoring of organic contaminants in aquatic systems has traditionally concentrated on the measurement of concentrations in edible fish and shellfish. Environmental effects of contaminants, however, can best be determined through analysis of food chain contamination with particular attention given to fish food organisms, primary producers (e.g. algae) and primary consumers. In this project levels of PCB in phytoplankton, zooplankton and ichthyoplankton throughout a 250 km stretch of the tidal Hudson River have been determined.

PCB concentrations in Hudson River plankton varied from below detection limits in downriver areas (N.Y. City, Lower Bay) to values > 15 ppm near Albany. The dominant PCBs detected were the dichlorobiphenyls through the hexachlorobiphenyls indicative of Aroclor 1016 and Aroclor 1254 in the environment. Experimental evidence, however, suggests that the isomer distributions seen in the river are not true indicators of contaminant materials, but artifacts of PCB volitalization, microbial degradation and selective deposition of PCBs in sedimentary sinks.

Although the major source of PCBs for the Lower Hudson is sediment transport through the Federal locks at Troy, contamination of the biota and the PCB isomer distribution point out alternative PCB sources of major significance. In general, PCB contamination of plankton was greatest near urban centers (e.g. Kingston, Poughkeepsie, and Tarrytown), where the discharge of wastewater may contribute PCBs to the system. Data are unavailable on actual PCB loads. However, estimates of PCBs in wastewater range as high as 20 ppm (dry weight), and sewage sludge may contain ~ 5 ppm (dry weight).

While plankton in the estuary are highly contaminated with PCBs, there exists no evidence for biomagnification in the system (i.e. all trophic levels of the presumptive Hudson River food web contain similar concentrations of PCBs). The distribution of PCBs in organisms is primarily a function of location in the estuary, rather than an organisms' trophic level. Despite this, the presence of PCBs throughout the food web implies the potential for significant transport from organism to organism; The generally high level of PCBs in estuarine organisms is maintained by food sources, as well as by water uptake.

PCB contamination in Hudson River plankton varies significantly with the time of year because of seasonal variations in particle transport, sediment scouring, and surface runoff. A positive correlation exists between PCB levels in Hudson plankton and river flows for the previous month at upriver stations. At stations further downriver, seasonal

effects are masked by sedimentation of PCB laden particles, and dilution with large volumes of sea water.

Isomer distributions of PCBs from environmental samples suggest a continual transformation of PCB source material. Despite the fact that most of the PCBs discharged into the system were Aroclor 1016, Aroclor 1254 dominates in many organisms at many stations. The ratio of 1016 to 1254 has been used to identify "new" PCBs in the system, and it was highest near urban areas and an ongoing dredging project. This implies that there exists a source of PCBs at these locations which is new, or which had only recently been exposed to ecological processes in the River system.

Analysis of PCBs in plankton from natural systems provides an excellent indication of PCB contamination. Planktonic organisms are capable of taking up PCBs in large quantities, storing them efficiently, and providing an indication of total system contamination without the confounding factors of migration and movement associated with monitoring PCB levels in fish.

Sponsors: N.Y. State Department of Environmental Conservation and Institute of Environmental Medicine

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Staff: J.C. Pizza, M.S., D.W. Bath, M.S., J.A. Hernandez, A.A.S. and S. Riordan

11. Lethal and Sublethal Effects of PCBs on Aquatic Organisms

Considerable controversy exists as to the sublethal ecological effects of polychlorinated biphenyls on aquatic organisms. In current studies, this laboratory is testing the effects of PCBs on respiration of planktonic crustacea and fish and attempting to determine the tissue partitioning of PCBs in fish, as indicators of potential ecosystem impact.

Extensive studies have been carried out of the respiration of *Gammarus tigrinus* to determine respiratory response to low concentrations of PCBs in the water and in the organism. Water concentrations during tests were ~ 1 ppb, and body burdens were ~ 0.5 ppm (dry weight). Overall, PCBs had a slightly negative effect on rates of metabolism (mg O_2 consumed per mg body weight per hour). However, the depressive effect was not statistically significant. Throughout the studies completed to date, age and size of the test organism did not have a strong effect on the respiratory response to PCBs, despite the fact that young

Gammarus are more susceptible to the lethal effects of PCBs than are adults.

Research with fishes (striped bass) has concentrated on determining the relative importance of PCB uptake from water or food in determining whole-body PCB levels. Fish have been exposed to 0.5 and 1.0 ppb PCBs in water, and to doses of up to 100 ng of PCB in food, administered by gavage. PCB uptake directly from the water was found to be a logarithmic function related to two factors, the area of the gill surface, and the ventilation rate of the gills (i.e. liters of water per hour passing over the gill). The efficiency of PCB removal during a single pass of water over the gill is under study but has not been determined. For larval and adult fish, water uptake of PCBs is source dependent. Steady-state conditions are attained over a period of more than 100 days, although the mathematical relationship of PCB uptake to fish size during the first 12 hours of uptake may provide an accurate estimate of final PCB concentration.

PCB uptake from food is a highly efficient process, and between 95-99% of the PCBs in food are assimilated by striped bass. Following assimilation, tissue distribution is highly predictable, and it differs significantly from tissue distribution following water uptake. During water uptake, PCBs partition to the gill, kidney, stomach, liver and muscle, in that order. Following uptake from food, the vast majority of PCBs go directly to the liver and to the gall bladder. Thus, fecal material contains high concentrations of PCBs, because of the excretion of bile into the intestine.

The processing of PCBs from food provides a clearance mechanism for organisms feeding upon PCB-laden food. When food is contaminated at low levels, PCBs from food are excreted rapidly in fecal material, and they do not contribute substantially to overall body burdens. However, when food is contaminated at high levels, the ability of the liver to clear PCBs is exceeded, and the contaminants enter the general circulation, adding large quantities of PCBs to the whole organism.

Data from environmental samples confirm this hypothesis. In the Hudson estuary, where fish food organisms may contain > 5 ppm PCBs, fish body burdens are exceedingly high, up to 50 ppm. The same species in the coastal N.Y. Bight region, however, with access to a food supply with $\leq$ 0.5 ppm PCBs has overall body burdens similar to that predicted from experiments with direct water uptake; i.e. $\sim$ 0.05 to 0.1 ppm PCBs.

Sponsors: N.Y. State Department of Environmental Conservation and Institute of Environmental Medicine

Principal Investigators: J.M. O'Connor, Ph.D., C.C. Lee, Ph.D. and T.J. Kneip, Ph.D.

Staff: J.C. Pizza, M.S., D.W. Bath, M.S., J.A. Hernandez, A.A.S. and S. Riordan

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12. Transport of PCBs in Coastal Marine Food Webs

Contaminated finfish and shellfish are the most likely vectors for the transport of chemical contaminants from aquatic ecosystems to man. The potential for marine food chains to concentrate organic contaminants either directly from the water (Bioconcentration) or from water and food (Bioaccumulation) can result in contaminant concentrations in seafood which exceed established FDA action limits. Such bioaccumulation has already rendered striped bass from the Hudson River questionable as a food resource.

This food web study is aimed at describing the dynamics of PCB transport in model food chains consisting of organisms which inhabit the sediments of the Hudson estuary and its associated coastal marine waters. The estuarine food chain consists of the sediments, a polychaete worm (*Neanthes succinea*) and an estuarine bottom feeding fish, the hogchoker (*Trinectes maculatus*). The marine food chain consists of marine sediment, *Neanthes succinea* (or *Nereis virens*) and the winter flounder (*Pseudopleuronectes americana*). Transport studies involve the use of ^{14}C-labelled PCBs, the mixtures of aroclor 1016 and 1254, and pure PCB isomers.

Sampling for hogchokers and *Neanthes* began in mid October, and preliminary uptake studies have begun.

Sponsor: U.S. Environmental Protection Agency

Principal Investigators: J.M. O'Connor, Ph.D. and C.C. Lee, Ph.D.

Co-Investigator: T. J. Kneip, Ph.D.

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13. Identification of Contaminants in Dredged Material from N.Y. Harbor

Sediments in the vicinity of New York City are highly contaminated with a variety of materials including PAHs, PCBs, chlorinated pesticides, toxic metals and other domestic and industrial wastes. There is much concern over the possibility that the disposal of contaminated sediments in the relatively clean ocean waters adjacent to New York will result in contamination of food organisms, with the eventual transport of toxic compounds back to man.

Sediments from seven contaminated sites in the Hudson River, the N.Y. Port district, and New Jersey Port district are being analyzed to determine the distribution of various contaminants in the region. Core samples from the ocean dumpsite have been taken to determine the stability of these contaminants in a dump zone which has been isolated by emplacement of a clean sand cap over the contaminated sediments.

Preliminary studies of contaminant concentrations, grain size, organic carbon content and metals content have begun. Most of the contaminated sediments have the major fraction of particles in the silt and clay range and high organic content. This suggests that levels of hydrocarbons, including PAHs and PCBs, will be high in the contaminated sediments. Predicted PCB concentrations, based upon preliminary data, range vary from 1.0 to 5.0 ppm dry weight. PAH levels could range from 0.5 to 100 ppm. No data are available, as yet, to differentiate sediments according to location.

Sponsor: U.S. Army Engineers, N.Y. District

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Staff: D.W. Bath, M.S., J.A. Hernandez and R. Califano, M.S.

14. Distribution and Abundance of Cadmium in Blue Crabs

The brackish waters of the Hudson River support the major New York State sport and commercial fisheries for blue crabs (*Callinectes sapidus*). A significant crab fishery extends as far upstream as Newburgh and Poughkeepsie, 65 to 70 miles above New York City. In the course of studying a localized instance of cadmium contamination at Foundry Cove on the Hudson River, it was noted that blue crabs from several sites in the Hudson contained high concentrations of Cd in the flesh and in various internal organs. High-level contamination was not restricted to the Foundry Cove area, where sediment cadmium values reached 10,000 ppm or more. Crabs from regions where sediment Cd values did not exceed 6 ppm contained Cd levels similar to those observed in crabs from Foundry Cove.

Analysis of various tissues from crabs in the Hudson has shown that the highest Cd levels occur in the hepatopancreas ("mustard"), a portion of the organism which is frequently eaten in steamed or boiled crabs and always eaten in soft-shell crabs. Hepatopancreas Cd levels may range up to 30 ppm and they could provide a dose to the consumer of up to 1 mg of Cd per crab consumed. Cd contamination is severe only in crabs from the brackish and freshwater portions of the River, at salinities less than 7-10 parts per thousand.

Current research is aimed at describing and characterizing the mechanisms whereby crabs accumulate large quantities of Cd in the hepatopancreas. It has been determined through laboratory studies that Cd in the hepatopancreas is bound to a protein with characteristics similar to those of mammalian metallothionine. The synthesis of large quantities of the protein can be induced by exposing crabs to low levels of Cd under laboratory conditions. A Cd-binding protein with similar characteristics (i.e., UV absorption at 250 nm, a molecular weight $\sim$ 10,000, and a high cysteine content) has been isolated from crabs caught in the field.

The studies have shown that protein binding is the dominant mechanism used by crabs to remove Cd from circulation in the hemolymph, and to maintain low Cd values in the somatic muscle, gonads, gut, etc. Recently it has been determined that a small amount of Cd is eliminated in the moulting process, but less than 15% of the Cd in the crab is sequestered in the shell and other body parts lost during a moult. Thus, the rather profound endocrinological and physiological processes which occur during moulting do not result in the mobilization and elimination of much Cd.

The tendency for crabs to concentrate Cd in the hepatopancreas in brackish waters appears to be of significance in determining the extent

to which persons who eat crabs are exposed to Cd. Crab muscle does not contain enough Cd to cause acute toxicity, since for the most part, Cd concentrations in muscle are relatively low (0.05 to 0.1 μg/g). However, if the hepatopancreas is consumed, the high Cd concentration in that organ and its large mass could cause a significant increase in Cd ingestion, and, possibly, acute intoxication. For some crabs, up to 1 mg of Cd may be present in the hepatopancreas (e.g. a 30 g hepatopancreas at 33 μg/g Cd may contain 1 mg total Cd). Even mean values of 8-15 μg/g represent a significant dose (240-450 μg/crab eaten), and the amount of Cd ingested in a single meal of 10 typical crabs could provide an acutely toxic Cd dose of 2.4-4.5 mg Cd. Even if acute doses do not occur, regular consumption of hepatopancreas from Hudson River blue crabs may increase Cd intake to values equal to or greater than the WHO maximum acceptable limits of ∿ 45,000 μg per year.

Sponsor: Institute of Environmental Medicine

Principal Investigators: J.M. O'Connor, Ph.D., and T.J. Kneip, Ph.D.

Co-Investigators: S. Garte, Ph.D.

Staff: A.M. Wiedow, M.S.

Publications:

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15. Environmental Health Aspects of Metals and Organic Contaminants in the New York Bight

The New York Bight and adjacent waters receive the domestic, industrial and agricultural waste from a population of more than 20 million people. These wastes contain large quantities of several important contaminants, including heavy metals, chlorinated pesticides, PCBs and polynuclear aromatic hydrocarbons. Once in the coastal marine system, many of these contaminants may enter marine food chains, and they have the potential to be concentrated in fish and shellfish products which are consumed by the human population. This project has as its objectives the collation of available data on toxic and potentially toxic compounds identifiable in the New York Bight ecosystem and the evolution of the potential for various contaminants to become transferred to human beings. As a result, this laboratory has studied studied several aspects of contaminant transport in marine systems, identified major sources and sinks of contaminants, and derived estimates of public health risk to human beings from consuming fisheries products from the Bight.

The major causes of contaminant transport of toxic metals in the Bight system are current driven sediment movements, dredging activities, and the disposal of dredged materials in the N.Y. Bight. Most of the metals load in the N.Y. region remains associated with deposited sediments. However, significant quantities of cadmium (Cd) and zinc (Zn) may be released from sediments or sewage sludges during dumping operations. Metals in the dissolved state, as well as particle-bound metals, may be accumulated by fishes and shellfish.

Simple estimates of bioconcentration factors (BCF) for metals provide virtually no insight into the ecological effects or the potential public health impact of metals in natural systems. Despite published estimates of BCF for Cd, Cu, Zn, Pb and Hg which may range up to 1×10^6, present body burdens of metals in Bight organisms are low, with BCFs ranging from 10^2 to 10^4. Thus, the levels of metals in food organisms from the Bight present no threat to public health.

The distribution and ultimate fate of toxic metals in the Bight system depend on a steady-state between metals loading, metabolism in organisms, and natural biochemical processes of metals inactivation (i.e. deposition, chelation, protein binding, etc). Transport mechanisms and transport rates of metals in the biota depend primarily upon food-chain phenomena. However, no evidence exists for stepwise increases of metal burdens at each level of the food chain.

Pesticide concentrations in the Bight ecosystem are low, reflecting the statewide trend of decreasing values of DDT, mirex, etc. in surface waters. However, the N.Y. metropolitan area is a significant source of DDT for the coastal marine system. The dumping of sewage sludge containing 2-3 ppm DDT accounts for an annual load of $\sim$ 800 kg of DDT entering the Bight and available to marine organisms. The accumulation of DDT in

marine food organisms, however, is small and presents no public health threat, since DDT concentrations in general are $\leq$ 100 ppb in the edible flesh of finfish, bivalve molluscs and crustacean shellfish.

The major source of PCBs for the Bight ecosystem is downstream transport from the Upper Hudson. Several alternative sources also exist in the Lower Hudson, primarily wastewater discharges in the vicinity of population centers. Approximately 6,000 kg of PCBs are deposited annually in the Bight ecosystem with dumped dredge material and sewage sludge. Much of this material is deposited in the Harbor from upstream sources and subsequently removed by dredges for eventual transport to the Bight.

Organisms in the Hudson River contain high concentrations of PCBs, from $\sim$ 1 ppm in zooplankton to $>$ 50 ppm in certain species of fish. The same or similar species in the coastal system contain much lower body burdens of $<$ 0.5 ppm PCBs. Concentrations of PCBs in fish and shellfish from the Bight are apparently determined by water-tissue partitioning, and they are not influenced by the dietary transport of PCBs. In the Hudson, however, dietary sources of PCBs are the major determinant of PCB body burdens in fish. Thus, fishes from the Hudson have been found to present a public health threat, while fishes and shellfish from the Bight region do not.

The ecological effects of long-term PCB contamination in the Hudson River/Bight system have not been determined. However, fisheries remain highly productive in the Hudson, and basic indices of ecological integrity (i.e., species diversity, primary production, and biomass) provide a basis for optimism.

Fifteen polynuclear aromatic hydrocarbons (PAHs) occur regularly in high concentrations in the Hudson/Bight system. PAHs are most abundant in sediments adjacent to docks, in areas of oil tanker lightering, and near sewage plant outfalls. Regular disposal of sewage sludge and dredge spoil in the Bight accounts for an annual load of more than 150,000 kg of PAHs to the system. The major sources of PAHs are combined sewer overflows, stormwater runoff and oil spills.

The commercial fish and shellfish of the N.Y. Bight contain relatively small quantities of PAHs, because most marine organisms are capable of either rapidly excreting most PAHs or metabolizing them to harmless compounds.

Sponsor: U.S. Department of Commerce, NOAA, Marine Ecosystems Analysis Project

Principal Investigators: J.M. O'Connor, Ph.D. and T.J. Kneip, Ph.D.

Staff: D.W. Bath, M.S., J.C. Pizza, M.S. and R. Collazo

Publications:

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16. Degradation of Polychlorinated Biphenyls by Fungi and Anaerobic Bacteria

PCBs in aquatic environments tend to accumulate in reducing or anaerobic sediments, and the principal source of human exposure to PCBs is through the consumption of PCB contaminated fish taken from these environments. Although many reports document the effect of PCBs on the metabolism of aquatic and terrestrial organisms, the metabolic degradation of PCBs itself has only recently received much attention. Published information suggests that while deposition in sediments and volatilization to the atmosphere may be major processes which remove PCBs from surface waters, microbial degradation may be a mechanism whereby PCBs can be eliminated from the environment.

Few data exist on the rate at which PCBs are being degraded in nature. Those that are available deal exclusively with aerobic bacterial systems, and they are not directly applicable to PCBs found in reducing or anaerobic sediments. However, there is evidence that chlorinated pesticides and other aromatic compounds can be degraded under anaerobic conditions and that the degradation of lower PCB isomers in environmental samples may be carried out primarily by anaerobic bactera. Little information is available on the role of fungi in PCB degradation, even though fungi are endemic to many aquatic ecosystems and play an important role in the recycling of organic matter.

The major objective of this study is to investigate PCB degradation by fungi and anaerobic bacteria, to determine if the potential for PCB elimination by these microorganisms exists in nature.

Fungi and anaerobic bacterial cultures have been established from selected aquatic environments (both PCB-free and contaminated) across the United States, and studies to screen these isolates for PCB-degrading activity have begun. Only those fungi and/or anaerobic bacteria which are able to grow in medium enriched with 0.05% Aroclor 1254 were selected for further study.

Much of the ongoing research is centered on the fungus (*Fusarium* sp.) and a culture of mixed anaerobic bacteria (designated MK-1), both of which were isolated from PCB-contaminated sediments of the Hudson River. Subsequent testing of other fungi and anaerobic bacteria for PCB degradation will follow procedures developed in the study of these two cultures.

Growth data derived from *Fusarium* and from MK-1 show that certain fungi and anaerobic bacteria can degrade Aroclor 1254. With the aid of ^{14}C-labeled Aroclor 1254 and the use of thin-layer chromatography and liquid scintillation counting techniques, $^{14}CO_2$ and other ^{14}C-labeled products (as yet unidentified) have been recovered from extracts of both cultures. The presence of these products is correlated with a decrease in the total PCB concentration in the respective cultures. Changes in the peak ratios of gas chromatograms of PCBs subjected to microbial activity were also observed, and they may indicate selective alteration of individual classes of PCBs, possibly by dechlorination.

$^{14}CO_2$ data in these experiments indicate that 0.1 and 0.01% of Aroclor 1254 was mineralized within one week by the *Fusarium* and by the MK-1 anaerobes, respectively. Although a 2-10% yield of ^{14}C-labeled metabolites of Aroclor 1254 has been recovered in 96-hr. experiments with the MK-1 anaerobes, similar data are not yet available for *Fusarium*. Work is continuing to obtain quantitative estimates of PCB utilization by *Fusarium* and by MK-1 bacteria, and to identify the products. Special efforts are being made to obtain a mass balance accounting for the ^{14}C-label used in the experiments.

The present studies are being expanded to include studies to show dechlorination. Microbial systems will be given a PCB containing 5 or 6 chlorines. If the PCB is dechlorinated, new PCBs containing 5 or fewer chlorines, detected by gas chromatography, would result. However, if the PCB is not dechlorinated, it would be observed unaltered in the gas chromatogram.

Sponsor: Monsanto Company

Principal Investigator: C.C. Lee, Ph.D.

Staff: R. Sansur, M.S. and J. Hernandez, A.A.S.

17. Determination of the Concentration, Distribution, and Sources of Radionuclides in the Hudson River Estuary

a. Beta/Gamma-Emitting Radionuclides

The long term goal of this research (initiated in 1964) is to develop a quantitative understanding of the fundamental biological, chemical, and physical factors associated with the transport and ultimate fate of various manmade radionuclides within the Hudson River Estuary.

During 1980, over 500 samples of fish, plants, water, and sediment were collected and assayed for the natural radionuclides, ^{40}K, ^{226}Ra, and ^{232}Th, and for the artificially produced fission and activation products ^{137}Cs, ^{134}Cs, ^{54}Mn, ^{60}Co, and ^{58}Co. Data analyses were performed using a 4" x 4" NaI(Tl) well crystal coupled to a 512 channel analyzer and a 97 cm^3 Ge(Li) detector coupled to a 4000 channel computer-based analyzer.

Because reactor operations have introduced measurable quantities of various radionuclides into the river ecosystem, the field sampling program has centered around the nuclear electric generating facility at Indian Point, 43 miles upstream from New York City. Sampling extends 35 miles upstream from the reactor site to Esopus, where essentially "background" concentrations are obtained for comparison with samples collected adjacent to and downstream from the reactor site.

Preliminary results for 1980 indicate that for ^{137}Cs (the nuclide of major dosimetric interest), concentrations in both surface and deeper sediments recently collected from the Indian Point vicinity appear to have reached equilibrium following the 1971 reactor release (~20 Ci).

The estimated yearly human radiation dose resulting from reactor releases of ^{137}Cs has been estimated, from models incorporating both shoreline exposure and exposure from consumption of Hudson River fish, to be approximately 0.1% of the yearly average natural background radiation dose rate previously measured.

b. Isotopes of Plutonium

The biogeochemistry of plutonium is currently being studied in an estuarine environment. A prospective study has been initiated in which the most probable current sources of plutonium for the estuary are being monitored on a continuous basis. Measuring stations, located just south of the Federal Dam in Troy, NY, and in the liquid effluent discharge canal of the Indian Point nuclear reactor complex, will provide continuous information about current inputs to the river of fallout and reactor-

derived plutonium arising from erosional processes occurring throughout the drainage basin, and possibly from low level releases of plutonium from the reactor complex.

A retrospective study is also in progress in which core samples, taken over the entire length of the lower Hudson, from Albany to New York Bay, and from the drainage basin are being assayed for ^{239}Pu, ^{238}Pu, ^{137}Cs, and ^{134}Cs to determine the total budget of the nuclides in the Hudson and its drainage basin. The rate at which these nuclides are moving through the estuary, the areas of greatest sediment radionuclide accumulation, and the temporal history of recent sediment deposition, using the radionuclides as tags for sedimentary processes, are also being examined.

Samples of fish, rooted vegetation, crabs, and plankton have recently been collected from the river, and they are being assayed for both plutonium and cesium isotopes. An assessment of the transfer of plutonium from water through the aquatic food chain to man is currently under investigation.

Results of an *in vitro* experiment designed to simulate the behavior of plutonium in a water-sediment environment as a function of time, salinity, pH, sediment particle size and mass, and water chemical composition indicate that, for Hudson River water, equilibrium partitioning of plutonium between the suspended and dissolved phase is reached rapidly (~1 hour). Once plutonium is bound to sediment, desorption will not occur with increasing salinity, as was noted for ^{137}Cs. The mean distribution coefficient (K_d = pCi/kg (suspended) ÷ pCi/l (dissolved)) for plutonium in Hudson water containing typical (10-60 mg/l) suspended loads of clay-sized sediment was $\sim 5 \times 10^5$. This value was found to drop severely only at unusually low pH's (i.e., 0-1) and where increasing dissolved organic matter was present (i.e., at 21 mg/l sodium humate, $K_d = 4 \times 10^4$).

Sponsor: Consolidated Edison Company of New York, Inc.

Principal Investigators: N. Cohen, Ph.D. and M. Eisenbud, Sc.D.

Staff: P. Linsalata, M.S., S. Schaffer, M.S. and L. Ayers, A.S.

Publications:

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18. Radionuclides in Enewetak Beach Coral Samples Collected in 1956

In 1956, Dr. Henry Hirshfield of the Department of Biology visited Enewetak Atoll to collect biological specimens during Operation Redwing, part of the U.S. nuclear weapons testing program. Of the specimens brought to the U.S., only beach coral samples have still remained available for analysis. The majority of these samples were collected from the fringes of test site craters, some of which had been made within several weeks of sample collection.

An extensive survey of the atoll was performed in 1973 by the U.S. Atomic Energy Commission as part of a pre-decontamination and pre-settlement action. A comparison of radionuclide concentrations in samples found during the 1973 survey with those collected in 1956 may allow estimation of the long-term dispersion and dilution that could take place in such an ecosystem.

Radionuclides found in the samples were ^{137}Cs, ^{60}Co, ^{155}Eu, ^{241}Am, and $^{238,239}Pu$, and the concentrations varied widely from crater to crater. No significant differences were seen between the concentration averages in the 1956 samples and those in the 1973 survey samples. This may be due to 18 additional megaton-range tests that occurred on the atoll between 1956 and 1973, which increased the concentrations over those in 1956.

The 1956 samples consisted almost entirely of coral or coralline fragments and foraminifera. Because these organisms have calcareous exoskeletons, they may actively take up radionuclides known to be bone-

seekers in man. If such uptake is observed in foraminifera, they may be useful as indicator organisms to determine whether an aquatic environment is contaminated with such radionuclides. To investigate this possibility, one of the more highly contaminated 1956 samples was separated into five fractions: three genera of foraminifera and two sizes of coral/coralline fragments. Analysis of these fractions is in progress.

Sponsor; U.S. Department of Energy, Contract No. DE-AS02-76EV-03382

Principal Investigator: N. Cohen, Ph.D.

Co-Investigator:. M. Eisenbud, Sc.D.

Staff: H. Hirshfield, Ph.D. and T. Rahon, B.S.

Publication:

Rahon, T.E., N. Cohen and H. Hirshfield. Radionuclides in 1956 Enewetak Beach Coral Samples. *Proc. of the 26th Annual Bioassay, Analytical and Environmental Conference*, Ottawa, Canada, October 14-15, 1980. In Press.

19. Responses of *Gammarus tigrinus* to Short-Term Exposure at Elevated Temperature and Chlorine-Produced Oxidant Stress

This research project has evaluated the lethal and sublethal responses of the estuarine crustacean amphipod *Gammarus tigrinus* Sexton, to acute exposure either to the physical stress of elevated temperatures, or to the chemical stress of chlorine-produced oxidants.

Estuaries are of primary concern in considering the actions of these stressors for two reasons. First, estuarine waters are highly productive, accounting for many of the U.S. fisheries. Secondly, high-density population centers are located near estuaries, and their presence maximizes the potential for impact by both thermal and chemical effluents.

Gammarus tigrinus epitomizes the truly estuarine crustacean. It has wide salinity tolerance, being able to reproduce in fresh or sea water, and it dominates the macrofauna in environmental samples collected from several estuarine systems. Its short generation time (~ 5 weeks at 22°C), its ready adaptibility to culture methods, and its susceptibility to thermal and chemical stresses make this amphipod species ideal for the study of both acute and chronic responses.

A device capable of generating stable thermal or chemical conditions in a flow-through design was utilized for exposures. Lethal responses to the stressors were established for the species through exposure of

newly released young amphipods to various temperatures or oxidant concentrations for durations of 3, 10, 30 and 100 minutes. For elevated temperatures, median lethal levels (LL50s) were related to duration of exposure (DE) in minutes as follows: LL50 = 40.2 - 2.43 log DE. For chlorine-produced oxidant stress, median lethal concentration (LC50), measured as milligrams per liter, was related to the duration of exposure as follows: log LC50 = 1.629 -1.116 log DE. After 10-min exposures adult animals demonstrated greater tolerance to thermal stress than did the young, but the reverse was true with chlorine-produced oxidants.

After sublethal exposures to the two stressors, metabolic physiology, behavior, growth, and reproduction were examined. Oxygen consumption rates of individual adult animals increased immediately following 10-min exposures but returned to normal within 24 hr. When the adenylate phosphate stores (ATP, ADP and AMP) of newly released young were extracted and assayed with a luminescence biometer, it was found that the ratios of the various adenylate species and the total adenylate concentrations changed only after exposure in the lethal range.

Two behavioral patterns were quantified, and their susceptibility to modification under stress was investigated. Motility was assessed before and after exposure for 10 min to each toxicant, and it was found that swimming ability was inhibited at a temperature 4° below the LL50 and at chlorine-produced oxidant levels an order of magnitude below the LC50. Precopulatory amplexus proved to be highly labile during exposure to temperatures more than 5° below the LL50, while at chlorine concentrations even greater than the LC50, males and females remained in amplexus.

The growth of newly released young was assessed at weekly intervals after an initial 10-min exposure to either ambient water, water at 37.0°C, or water with 0.5 mg/l chlorine-produced oxidants. After four weeks the total lengths and weights of the stress-exposed animals were less than those of the controls. The numbers of young released by individual female gammarids were also observed for one moulting period prior to exposure, and for two moulting periods after exposure for 10 min to the same three conditions. Embryo toxicity was identified only in the thermally stressed group. Latent effects of the toxicants were observed only in the chlorine-produced oxidant group, which had reduced numbers of offspring.

The results indicated a variable response by *G. tigrinus* to acute physical and chemical stress. The amphipods rapidly detected thermal increases but were insensitive to elevated levels of chlorine-produced oxidants. With temperature stress, the immediate responses were increased respiration rates and embryo toxicity. Recovery followed within 24 hrs for respiration and within a reproduction cycle for female fecundity. After chlorination stress, the immediate reaction was an increased respiratory rate, followed by a 24-hr recovery. However, persistent latent effects upon the reproductive capacity of females were observed. Nominally sublethal exposures were demonstrated to have chronic impacts upon the growth and maturation of these amphipods.

Sponsor: U.S. Nuclear Regulatory Commission (through the New York State Energy Research and Development Authority)

Principal Investigator: J.M. O'Connor, Ph.D.

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Publications:

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20. The Transport of Thorium at the Morro do Ferro: An Analog for Modelling the Geochemical Transport of Plutonium from Nuclear Waste Repositories

The first year of a Brazilian-U.S. collaborative study of the mobilization of thorium from the Morro do Ferro, a hill in the center of the Pocos de Caldas plateau in the State of Minas Gerais, has been completed. The hill contains an estimated 20,000 metric ton (MT) deposit of thorium near its highly weathered summit. Rainfall has averaged about 170 cm/yr, and drainage is into an easily accessible self-contained basin. The deposit also contains uranium and rare earths, and plans have been made to study these elements during the second year of research. Because of similarities in the chemistries of thorium and plutonium under environmental conditions, this study should further our understand-

ing of how plutonium would be mobilized from a nuclear waste repository that has been breached by geological or other processes.

Mobilization is being studied both by measuring the thorium concentration of stream water draining the hill, and by analysis of dated cores from sedimentary deposits in the drainage basin. Hydraulic flow from the ore body is into a small stream that originates at the base of the hill. A flume has been constructed which is being equipped with a continuous-flow recorder and a system for sampling the water at a rate proportional to the stream flow.

During the past year, duplicate monthly samples were collected at four locations. Eh and pH measurements were made, and the samples were then filtered through 0.45 μm Millipore filters. The filtrates are acidified, reduced in volume from 20 to about 0.25 l, and shipped to New York for thorium analysis by alpha spectrometry.

Analysis of thorium in the stream water has proven to be difficult because of the low concentrations that exist and the inherent complexitites of the procedure. The mean total thorium concentration (dissolved plus suspended particulates) in 22 samples of stream water collected at intervals during the past year was 0.52 ± 0.64 μg/l. The standard error of the mean was 0.14 μg/l. As a first approximation, the estimated annual mobilization rate of the thorium deposit has been calculated to be 5.8×10^{-8} per year. The average life of the thorium deposit, assuming the present rate of mobilization, would thus be about 17 million years. Analyses of cores collected from sedimentary deposits downstream from the hill by gamma and alpha spectrometry, and by a system of x-ray fluorescence are currently in progress.

It has recently been estimated that the total U.S. inventory of ^{239}Pu by the year 2050 will be 1600 MT, based on a once-through fuel cycle, and 24 MT if the plutonium is recycled. Assuming that 1600 MT of plutonium were to be stored under conditions comparable to those at Morro do Ferro, the thorium measurements made to date suggest that the stream water would meet the International Commission on Radiation Protection plutonium limits for human consumption.

Sponsor: U.S. Department of Energy, Contract No. DE-AC97-79ET46606

Principal Investigator: M. Eisenbud, Sc.D.

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Staff: G. Laurer, Ph.D., N. Cohen, Ph.D., T. Kneip, Ph.D., R. Ballad, Ph.D., W. Lei, M.S., J. Furfaro, B.S., M. Carlos, B.S. and M. Vale, B.S.

Publication:

Eisenbud, M., T.L. Cullen, K. Krauskopf and E. Penna Franca. The mobility of thorium from the Morro do Ferro. Progress Report through September 30, 1980 for the U.S. Department of Energy and Comissao Nacional de Energia Nuclear, November 10, 1980.

21. Qualitative and Quantitative Analysis of Elemental Concentrations of Morro do Ferro Soils, Using X-Ray Fluorescence Spectrometry

Of the various analytical techniques available for analysis of the elemental content of Morro do Ferro soils, X-ray fluorescence (XRF) spectrometry has several unique aspects which make its use generally more favorable than others. Wet chemistry is not required, sample size is minimal, and the analysis is non-destructive and capable of detection to desired levels, i.e., 10 to 20 ppm for thorium. Furthermore, with present-day, solid state detectors, not only thorium and uranium, but the rare earths and other elements such as Fe and Zr, can be analyzed simultaneously.

The XRF system consists of a radioactive source for excitation, a target, a detector, and a multichannel analyzer with accompanying electronic components. The source is fabricated in annular form to maximize the intensity of the incident radiation. The energies required to ionize the K-shells of thorium and uranium are 110 and 116 keV, respectively. The only radioisotope with a reasonable half life able to ionize the K shell electrons with acceptable efficiency is ^{57}Co. Therefore, ^{57}Co is currently being used as the excitation source for thorium and uranium. To minimize calibration problems, ^{57}Co is also used as the excitation source for the lanthanides and neighboring elements of lower atomic number. The target consists of the sample positioned in a holder. Sample preparation consists of crushing, sieving (200 mesh), mixing with an organic binder (methocel) and then pelletizing in 30 mm diameter aluminum cups at 25 tons. By using a binder, the solid integrity of the pellet is preserved, preventing sample loss and potential detector contamination.

The high purity germanium, 80 mm^2 detector has an energy resolution of 484 eV FWHM at 122 keV and an active depth of 10 mm. The energy resolution of the detector allows resolution of the K_{α_2} (94.6 keV) emission line of uranium and the K_{α_1} (93.3 keV) emission line of thorium. The development of the overall XRF analytical procedure has been facilitated by the acquisition of a versatile computer-assisted multichannel analyzer system. This system was acquired with two data reduction programs -- Super ML, a background correction using a digital filter and multiple-least-squares fitting routine, and the Rasberry-Heinrich correction program to account for matrix absorption and interelement effects.

Optimization of the XRF system, for analysis of elemental content of the Morro do Ferro soil, has been achieved by the following considerations:

1. Self-absorption effects due to differences in matrix density and heterogeneity are minimized by using K-shell excitation of the thorium and uranium elements.

2. The geometrical relationship between the source, sample, and detector is set up to maximize the incidence photon flux and to minimize the spectral interference due to source photons backscattered from the sample.

3. The signal-to-background ratio for thorium is optimized by maximizing (net counts)^2/background ratio vs. the width of the peak in keV. The maximum point gives the optimal energy interval of the peak. This procedure has reduced the lower limit of detection.

4. The current lower limit of detection for thorium of 62 ppm, using a 10 mCi ^{57}Co source and a 1000 second count time, will be reduced to approximately 10 ppm by using a 40 mCi ^{57}Co source.

The first technique used for calibrating the thorium analyses is based upon the ratio of the net area under the ThK_{α_1} and ThK_{β_1} peaks to the area under the ^{57}Co (122 keV) Compton backscatter peak. This method has been validated by the use of known standards, and it can be used for quantitative analyses of thorium concentrations of Morro do Ferro soil samples. Results using this procedure have been verified by independent gamma and alpha spectrometric analysis. The current approach is to use the Super ML and the Rasberry-Heinrich correction programs. The Super ML program performs a multiple least-squares fit and generates K ratios, which are the ratios of the background corrected intensity of an X-ray line from the specimen to the corresponding corrected intensity for each element to be quantitated. Single element standards have been prepared for the Super ML program. The Rasberry-Heinrich matrix correction program requires mixed standards of varying concentrations which "closely" approach the composition of the unknown. Rasberry-Heinrich mixed standards of various levels of all the elements above have been prepared. The program corrects for interelement absorption and fluorescence effects and yields corrected concentration values for an unknown sample. Preliminary results indicate that the two data reduction programs will be adequate for simultaneous quantitation of the elements listed above.

Sponsor: U.S. Department of Energy, Contract No. DE-AS02-78EV-04957

Principal Investigator: G.R. Laurer, Ph.D.

Co-Investigators: M. Eisenbud, Sc.D. and N. Cohen, Ph.D.

Staff: James Furfaro, B.S. and Marcia Carlos, B.S.

IV. DESCRIPTION OF TEACHING PROGRAM

A. ENVIRONMENTAL HEALTH TRAINING PROGRAM

This section reports on the teaching activities of the Institute in the academic year from September 1, 1979 to August 31, 1980. Both graduate and post-doctoral training are emphasized. In various kinds of association with the Institute there were 89 graduate students. Approximately 65 can be considered to have been in full time training, both didactic and research apprenticeship. Twenty-five different graduate courses were given for formal credit. This level of teaching activity has been stable for several years.

Government training grants supported nineteen pre-doctoral and ten post-doctoral trainees. Three grants from the National Institutes of Health were in inhalation toxicology, environmental oncology, and lung defense mechanisms. The National Institute of Occupational Safety and Health is funding one training grant in Occupational Hygiene as a part of a larger consortium in the metropolitan area. A fifth grant from the Department of Energy in energy-related health problems was phased out during this past year. Several other mechanisms giving financial support to students are available including research and teaching assistantships and foreign and industrial fellowships. The above awards supported a total of 28 graduate students and 10 post-doctoral trainees.

Approximately 90% of the graduate students are enrolled in Ph.D. programs. The other 10% are enrolled in a master's program in occupational hygiene, which is the only specialty in which terminal master's candidates are accepted. The Institute has collaborative arrangements with the basic science departments at Washington Square and at the Medical Center, by which the student can matriculate formally in one of those departments and do thesis research under the supervision of a member of the faculty of the Institute. When this is not appropriate, or when the student does not want to emphasize a particular basic discipline, matriculation is in the Interdepartmental Program in Environmental Health Sciences. This is a separate academic unit of the Graduate School of Arts and Science, administered and directed by the Institute. About two-thirds of the graduate students are in the Program in Environmental Health Sciences.

For all students, the Institute provides courses in environmental health specialities and a perspective over the area of environmental health. The various types of matriculation allow for different amounts of related graduate study in a basic science. Student programs are available as guidelines in the areas of environmental toxicology, occupational-environmental hygiene, environmental epidemiology, and environmental radiation. Individual programs are adjusted to the specific backgrounds and goals of the students, with the Graduate Steering Group providing guidance and oversight for all students.

To help students choose their courses and research direction, temporary placements are given to them in one of the laboratories when it is practical to do so. This introduction to research is also continued in two seminar series: one is given by and for the students who present their own research work at the Institute, and the other is a formal weekly seminar series by invited speakers who give a broader and more advanced perspective on current research.

As of June 1980, seventy Ph.D.'s had been awarded through the Institute. In 1979-1980, Ph.D.'s were awarded to:

Fuad Amsyari - Thesis: *Occupational Radiation Risks Among Nurses Working in a Hospital Setting*

Passaporn Chittaporn - Thesis: *The Development of a Continuous Monitor for the Measurement of Environmental Radon*

Marc Halpern - Thesis: *Measurement of Particle Retention in the Lungs of Donkeys Using a Remanent Magnetic Field Technique*

David McCormick - Thesis: *Influence of Retinyl Acetate Administration Schedule on Inhibition of Rat Mammary Carcinogenesis*

Stephen Meyn - Thesis: *Inhibition of SOS Function by Antipain and λ Repressor: Evidence for Control of the SOS System by Proteolysis.*

As of June 1980, ninety-five master's degrees had been awarded. In 1979-1980, master's degrees were awarded to:

George Karron - Thesis: *The Pulsed Aerosol Delivery System (PADS): A Programmed Device for the Injection of Boluses During Electronically-Controlled Breathing Cycles*

Robin Lindenboom - Thesis: *The Effect of Atmospheric Pressure on the Collection Efficiency of a Passive Diffusion Dosimeter*

Lynn A. McFadden - Thesis: *Comparative Morphometry of the Upper Tracheo-bronchial Tree in Six Species*

Mark Palmieri - Thesis: *The Effects of Size, Sex, and Diet on the Composition of the Estuarine Amphipod Gammarus tigrinus in Laboratory Culture*

Alan Zeccardi - Thesis: *An Interrupter Method for the Measurement of Total Respiratory Resistance in Spontaneously Breathing Small Animals*

The graudate students having a traineeship or other type of fellowship award were:

Fuad Amsyari	James Green	Corrinne Martinelli
Nesrine Baturay-Smith	Howard Greenberg	John Mignano
James Butler	Andrew Hood	Maria Morandi
Passaporn Chittaporn	Philip Hunt	Andrey Nikiforov
Alice Dempster	Eileen Ivasaukas	Ted Rahon
James Dietrich	Karen Koenig	Gary Rosenthal
Jerry Formisano	Joyce Lipsztein	Michael Rozen
Dana Friedman	Robert Mallon	Gary Schwartz
Diana Galer	Randy Mersky	Thomas Vollmuth
		Alfred Wiedow

Post-doctoral trainees were:

Haw Huang Cheng, Ila Cote, Stephen Daniel, John Morris and Janice Teal in Inhalation Toxicology.

Lakshmi Natarajan-Atchison, Gail Theall and Jonathan Yavelow in Environmental Oncology.

Frank Rosenthal and Joel Silverman in Lung Defense Mechanisms.

ADVISORY COMMITTEE TO THE INTERDEPARTMENTAL PROGRAM IN ENVIRONMENTAL HEALTH SCIENCES, GRADUATE SCHOOL OF ARTS AND SCIENCE

R. Albert, M.D. (Environmental Medicine)
B. Altshuler, Ph.D. (Environmental Medicine)
B. Budick, Ph.D. (Physics)
M. Eisenbud, Sc.D. (Environmental Medicine)
N. Geacintov, Ph.D. (Chemistry)
H. Hirshfield, Ph.D. (Biology)
L. Kaufman, Ph.D. (Psychology)
N. Nelson, Ph.D. (Environmental Medicine)
D. Netzer, Ph.D. (Public Administration)
A. Upton, M.D. Chairman (Environmental Medicine)

GRADUATE STEERING GROUP OF INTERDEPARTMENTAL PROGRAM IN ENVIRONMENTAL HEALTH SCIENCES

B. Altshuler, Ph.D., Chairman
R. Albert, M.D.
F. Burns, Ph.D.
N. Cohen, Ph.D.
H. Hirshfield, Ph.D.
R. Jaeger, Ph.D.
M. Lippmann, Ph.D.
R. Shore, Ph.D.

B. MEDICALLY-CENTERED TEACHING ACTIVITIES

The Institute offers electives to undergraduate medical students in their first, second, and fourth years. Through these electives, they can participate in clinical and epidemiological studies of environmental and occupational agents which cause disease. A formal lecture course in epidemiology is given to second year classes, where a major part is devoted to chronic diseases and diseases caused by environmental agents.

A ten day full-time course in environmental health for physicians and allied professionals working in the area is given each June in the Post-Graduate Medical School and accredited for continuing education. It covers the major current problems and presents the fundamental background. This year attendees included 16 physicians, 6 industrial hygienists, and 4 other health professionals.

Stephen Meyn, an M.D.-Ph.D. fellow completed his dissertation (title given above) in the Laboratory of Biochemical Toxicology.

V. PROFESSIONAL STAFF

A. FULL-TIME STAFF

Arthur C. Upton, M.D., Professor, Director, Institute of Environmental Medicine, Chairman, Department of Environmental Medicine

Roy E. Albert, M.D., Professor, Deputy Director, Institute of Environmental Medicine, Vice-Chairman, Department of Environmental Medicine, Director, Laboratories of Experimental Medicine and Inhalation Toxicology and Carcinogenesis and Interlaboratory Epidemiology Unit

Bernard Altshuler, Ph.D., Professor, Associate Director, Institute of Environmental Medicine, Director, Biomathematics Laboratory

Sidney Belman, Ph.D., Professor

Fredric Burns, Ph.D., Professor

Merrill Eisenbud, Sc.D. (Hon.), Professor, Associate Director, Institute of Environmental Medicine, Director, Laboratory of Environmental Studies

Henry Hirshfield, Ph.D., Professor, Biology

Theodore J. Kneip, Ph.D., Professor

Morton Lippmann, Ph.D., Professor, Director, Aerosol and Inhalation Research Laboratory

Norton Nelson, Ph.D., Sc.D. (Hon.), Professor

Edward D. Palmes, Ph.D., Professor, Associate Director, Institute of Environmental Medicine, Director, Toxicology Laboratory

Bernard Pasternack, Ph.D., Professor, Director, Laboratory of Biostatistics and Epidemiology

Walter Troll, Ph.D., Professor, Associate Director, Institute of Environmental Medicine, Director, Laboratory of Biochemical Toxicology

Benjamin L. Van Duuren, Sc.D., Professor, Associate Director, Institute of Environmental Medicine, Director, Laboratory of Organic Chemistry and Carcinogenesis

Naomi Harley, Ph.D., Research Professor

Alvin Segal, Ph.D., Research Professor

Schwartz (Laboratory of Biostatistics and Epidemiology) has undertaken a health survey of the population of Staten Island. The basis for the survey is the fact that for the past 50 years, Staten Island has led the other boroughs of New York City in death rates for respiratory cancer and for other respiratory diseases, such as emphysema, bronchitis, and asthma.

A recent City-wide Health District analysis of age-adjusted death rates due to lung cancer showed that the districts with the highest rates over the past two decades lie in the path of the prevailing wind flow from the industrial complexes in New Jersey. The current study is designed to test the hypothesis that the high respiratory death rates observed in Staten Island are due to environmental factors which come from New Jersey, or from some other area outside the island. A team of interviewers and analysts has been assembled, and a questionnaire has been developed and field tested. A sampling plan for the Borough is now being developed, and it is expected that the full study will be completed in 3 to 5 years.

Lippmann, Lioy (Laboratory for Aerosol and Inhalation Research), Pasternack (Laboratory of Biostatistics and Epidemiology), Speizer and Fife (Harvard Medical School) studied the effects on children of exposures to summertime haze episodes in a non-urban western Pennsylvania community. Hazy air masses containing relatively high concentrations of ozone and sulfuric acid mist generally pass across the northeastern U.S. several times each month during the summer, and the highest pollutant concentrations can usually be found in western Pennsylvania.

Respiratory mechanical function measurements were made over a two-week period on 85 children, aged 9-13, who were enrolled in a summer day camp program. Ozone and other criteria pollutant concentrations and visibility measurements made by nearby state monitoring stations, and by aircraft and satellite monitoring platforms operating in the Persistent Elevated Pollution Episodes campaign of the U.S. EPA, are being analyzed to obtain exposure indices. In addition, a field team collected 6 hour aerosol samples on treated quartz filters, which are now being analyzed for H^+ concentration. They also operated a continuous flame photometric sulfur monitor which was modified by Husar of Washington University to measure H_2SO_4 concentrations over 15-minute intervals. The pollutant concentrations and the respiratory mechanical function effects data are being reduced to a computer-compatible form for analysis of association.

Schwartz (Laboratory of Biostatistics and Epidemiology) and Ferrara (Department of Pediatrics) are investigating the effects of infant transport on the survival of premature infants. There is a national trend toward the development of infant-transport systems for urban and rural areas, to enable premature children to be brought to centers of primary care for medical treatment. There is little or no information, however, on the effects of travel on the subsequent health of the children, and there is some suggestion in the literature that certain physiological indices, such as blood oxygenation, may deteriorate during travel.

The current study involves the New York City Health Department and 58 hospitals which will provide records of cases and controls. A large subject population is available since there are approximately 110,000 live births each year in the City, of which approximately 10% are premature. The development of the necessary questionnaires has been completed, the basic computer program library has been assembled, and data collection and processing have begun.

Pasternack, Shore and Green (Laboratory of Biostatistics and Epidemiology) have examined the relationship between aircraft noise levels and the percentage of students reading below grade level in all of the elementary public schools in Brooklyn and Queens. The percentage of students reading below grade level during the period of 1972 to 1976 was regressed on racial, socioeconomic, educational, and noise level variables for each school. Schools were assigned noise exposure scores based on Noise Exposure Forecast contours for the New York City airports. These exposure scores had a rank correlation of 0.95 with noise level measurements. The equivalent sound level at these measurement locations varied from 55 to 68 decibels. Regression coefficients, adjusted for confounding factors, indicated an additional 5% of the students in the noisiest schools read at least one year below grade level, with 95% confidence limits from 2% to 8%. In general, the percentage of students reading below grade level increased linearly with increasing aircraft noise level.

Altshuler (Biomathematics Laboratory) has continued mathematical and statistical studies of carcinogenesis. The major goal of the work is to interpret animal and human data to obtain quantitative implications for human risk at levels of exposure that may be proposed for environmental standards. The lack of empirical data at these levels requires a theoretical approach, despite the absence of a firm scientific base on which to operate. One phase of this work is a study of the inferences that can be made from multi-stage models of carcinogenesis. In this study, the time patterns of excess incidence with various types of exposure are simulated under a general set of plausible assumptions. A second phase deals directly with situations in which uncertainties cannot be evaluated by a completely objective methodology. Under this approach, Bayesian procedures are used to interpret sparse data for which asymptotic theory is not applicable. In more extreme situations, it may be desirable to introduce degree-of-belief probabilities as measures of informed judgment about poorly determined quantities.

Pasternack, Shore and Dubin (Laboratory of Biostatistics and Epidemiology) have continued work on the development and application of statistical, mathematical and epidemiological methods. Methods have been explored for analyzing the binary response data that arise in toxicological experiments involving littermates, and methods have been examined for analyzing data from comparative carcinogenesis experiments based on multiple times to tumor. Work has also been completed on the application of group sequential methods to epidemiologic studies of disease.

Work has also continued on the biomathematical modeling of the benefits and risks of screening for disease. In particular, a nonparametric life-table approach has been developed which will account for the radiation hazard associated with mammography. The method will permit a flexible characterization of incidence and mortality as functions of the number of screenings.

6. Research in Environmental Pollution and Ecology

Studies in environmental pollution and ecology cover a variety of research areas ranging from the chemical and radionuclide constituents of air masses to pollution of the aquatic environment. In the following reports, particular emphasis has been placed upon air pollution and upon the chemical contamination of the Hudson River and the coastal waters into which the river empties. The latter work is important since this water system is an outstanding laboratory for the study of pollutant transport and bioaccumulation processes in general and knowledge obtained in this laboratory is readily transferred to other water systems. Furthermore, the aquatic life of the Hudson estuary and the coastal marine environment is the focus of an extensive commercial fishing industry, and millions of people are exposed to food products taken from these waters. It is therefore essential to understand and to attempt to control factors which have a negative impact on this valuable resource.

Cohen, B., Lippmann (Laboratory for Aerosol and Inhalation Research) and Harley (Laboratory of Biostatistics and Epidemiology)) are investigating the reliability of current air sampling procedures for the determination of inhalation exposures. It has been reported that exposure estimates at a beryllium production facility determined by the method of time-weighted averages differ from those measured with full-shift personal lapel monitors by a factor of 2 to 3. The portion of the discrepancy between exposure estimates due to sampling device characteristics and to the aerosol characteristics and spatial distribution is being studied to determine which sampling method provides the best estimates of the true inhalation exposure.

The effects of flow rate measurement, filter efficiency and inlet characteristics have been evaluated and facilities have been developed to investigate the effects of the electric fields generated by personal monitors and protective clothing on the sampling process and on the aerosol distribution. A dust room was designed and built to study the question of dilution of the aerosol by the sampling procedures, and comparative measurements at the nose, lapel, and forehead are in progress to determine the relative aerosol concentrations at these locations.

Preliminary field measurements with a real-time aerosol monitor (RAM) have characterized the temporal variations in the breathing zone of a worker during a metal casting cycle, and a portable device incorporating 4 RAMS is being developed for detailed field monitoring of both temporal and spatial variability in aerosol concentration.

Kneip, Daisey (Laboratory for Environmental Studies), Lippmann and Lioy (Laboratory for Aerosol and Inhalation Research), have continued studies of air quality in New York City. Mallon (Laboratory for Aerosol and Inhalation Research) has used data for winters and summers from 1972-1976 to develop seasonal regression equations that identify the principal sources of pollution. Through these studies, it has been

found that soil dust is the most significant source of total suspended particulate matter (TSP) in summer, and oil burning for space heating is the largest source in winter.

After almost 15 years of steady improvement in air quality, an unanticipated increase has been observed in winter vanadium and TSP concentrations. The patterns observed in suspended particulate mass and composition indicate that fuel use and sulfur emissions have followed predictable patterns. The ash and vanadium contents of heating oil, however, appear to have increased, and data are being sought to test the hypothesis that fuel oil sources for the New York City area have changed since 1978.

Daisey, Kneip (Laboratory for Environmental Studies) and Lippmann (Laboratory for Aerosol and Inhalation Research) have continued studies of the sources of airborne particulate matter in New York City through the development of linear multiple regression models for the source apportionment of particulate organic matter (POM) and polycyclic aromatic hydrocarbons. Using these models, it has been possible for the first time to obtain estimates of the contributions of various sources to airborne concentrations of POM. The models indicate that 40% (3 $\mu g/m^3$) of the POM in respirable particles originates from oil-burning sources, while 19% (1.4 $\mu g/m^3$) derives from automobiles. Fifteen percent (1.1 $\mu g/m^3$) of the POM is comprised of soil-like particles. Preliminary models for benzo(a)pyrene indicate that 70% of this compound originates from oil-burning sources in the winter months.

Daisey, Kneip and Hershman (Laboratory for Environmental Studies) have continued studies of N-nitrosamines in particulate matter in the New York City atmosphere. Analyses of the seasonal variations in the concentrations of nitrosamines which have correlated the concentrations to those of vanadium, indicate that oil-burning for space heating is an important source of these compounds. The specific chemical identities of the N-nitrosamines involved have not been established, but clean-up and fraction-collection procedures are being developed for mass spectral analysis. In the meantime, further work has been carried out on the risk evaluation of the N-nitrosamines, where benzo(a)pyrene is being used for comparison.

Mukai (Laboratory for Biochemical Toxicology) and Daisey (Laboratory for Environmental Studies) have conducted investigations into the nature and sources of biologically active compounds in seasonal aerosol samples collected in New York City. The results indicate that a significant fraction of the mutagenic activity can be attributed to fuel oil consumption for space heating during the fall and winter seasons. Other sources of mutagenic activity which do not exhibit strong seasonal patterns, such as transportation, must also be involved, however, because biologically active materials were observed in the aerosol during summer as well as winter. High pressure liquid chromatographic profiles of the moderately polar dichloromethane-soluble fractions of airborne particles

are now being examined on a seasonal basis to determine whether qualitative and/or quantitative differences can be observed. If significant differences are seen, appropriate fractions will be isolated and subjected to mutagenicity testing and chemical characterization.

Lioy, Schwartz (Laboratory for Aerosol and Inhalation Research) and Daisey (Laboratory for Environmental Studies) have examined the effect of variations in sampling duration upon the reported concentrations of particulate organic matter (POM) collected on glass fiber filters. The results have shown that the apparent POM concentrations of cyclohexane and dichloromethane extractables are higher when sampling is carried out for 24 hours than when it is done for 6 hours. However, there is a shift from more volatile to less volatile constituents in the cyclohexane extractables with increased sampling time.

Kneip (Laboratory for Environmental Studies) and Van Duuren (Laboratory for Organic Chemistry and Carcinogenesis) have obtained transformed cells in experiments using a cocarcinogenic protocol for cell transformation with BALB 3T3/C and Wistar-38 cell lines. Preliminary studies have demonstrated growth of the transformed cells in soft agar and a more detailed characterization of the system is in progress.

Daisey, Kneip Laboratory for Environmental Studies), Lioy and Lippmann (Laboratory for Aerosol and Inhalation Research) have initiated studies of the transport of particulate organic air pollutants into the New York City area from other regions. Variations in the atmospheric concentrations of polycyclic aromatic hydrocarbons, sulfate, and ozone measured during the New York Summer Aerosol Study of 1976 were examined and compared with known air mass trajectories during the period. The data suggest that transport of aerosol from the coal-burning midwest contributes significantly to the atmospheric concentrations of polycyclic aromatic hydrocarbons observed over New York City under certain meteorological conditions.

Daisey and Kneip (Laboratory for Environmental Studies) have continued studies of the rates of degradation of polycyclic aromatic hydrocarbons adsorbed onto various substrates, under simulated environmental conditions, and the products that result from the degradation. In the past year, a method was developed to adsorb pyrene onto various substrates and to extract and analyze both undegraded pyrene and its degradation products.

The degradation of pyrene adsorbed on glass and on fly ash appears to follow a pseudo first-order rate law and to depend upon the presence of oxygen in the reactor, but not UV radiation. The half-life of pyrene adsorbed on fly ash is approximately twice as long as that of pyrene adsorbed on glass. Degradation products identified to date include the pyrene diones, diols, and bipyrene.

Lioy, Lippmann (Laboratory for Aerosol Research and Kneip (Laboratory for Environmental Studies) have initiated a study to develop ways to measure and characterize the acidic fraction of the atmospheric aerosol. The strong acid component in the form of the hydrogen ion (H^+) was measured using a calorimetric technique. *In situ* measurements of H_2SO_4 and all of the ammonium salts of the atmospheric aerosol were made with a fifteen minute resolution, and the sulfur species were distinguished through thermometric volatilization of the individual compounds. Measurement of the sulfur content was accomplished with a flame photometric detector.

Lioy (Laboratory for Aerosol and Inhalation Research) and Kneip (Laboratory for Environmental Studies) have characterized the winter and summer distribution and origin of airborne sulfur compounds in New York City for the period 1972 through 1979. The sulfate fraction of the atmospheric aerosol was found to be highest in the summer, while the highest SO_2 concentration occurred in the winter. This pattern is consistent with the results of previous studies which showed that most of the summer sulfate was attributable to regional sources. The most significant sources of the ambient SO_2 and sulfate in New York City in the winter are local residential and commercial space heating.

Daisey (Laboratory for Environmental Studies) has completed a study of airborne particulate organic matter (POM) and polycyclic aromatic hydrocarbons (PAH) in the Arctic aerosol in cooperation with investigators from the University of Rhode Island. The study showed that concentrations of POM, PAH, and ^{210}Pb were 2-10 times higher in March than in August at Barrow, Alaska, a remote site in the Arctic. Seasonal differences in the concentrations of these aerosol species, and in the meteorology of the area, as well as the presence of fly ash in the samples collected in March, indicated that the principal source of particulate organic species during this period was fossil fuel combustion. The results imply that there was long-range transport of these materials, since there were no local sources, and that the atmospheric life-times of the components must be long.

Lioy, Lippmann, Mallon (Laboratory for Aerosol and Inhalation Research) and Kneip (Laboratory for Environmental Studies) have used statistical analyses to show that there is a significant association between sulfate concentrations, measured at 10 sites in the northeastern U.S. during the summertime, and ozone concentrations measured at the same time. Significant correlations were observed between sulfate and ozone levels when measurements of the two were made on the same day, or when the ozone measurement was made one or two days prior to that of sulfate. A new technique for displaying the data which is called "isopleths of statistical significance" has also been developed. In addition, an advanced technique for representing a particular location as both a source and a receptor has been developed.

Cohen, N. and Ralston (Laboratory for Environmental Studies) are concluding investigations of the magnitude and organ distribution of fallout plutonium in human tissues. Initial studies were conducted on autopsy tissue samples obtained from residents of New York City. A radiochemical procedure, specific for the extraction of plutonium isotopes, was developed and state-of-the-art alpha spectroscopic methodology was employed for final detection. In those studies, 57 cases were analyzed giving median $^{239,240}Pu$ values of 0.37 pCi/kg for liver, 0.25 pCi/kg for vertebrae, 0.11 pCi/kg for T-B lymph nodes, 0.055 pCi/kg for lung, and 0.050 pCi/kg for kidney. Thirty-four additional cases, ten from Grand Junction, Colorado and 24 from Washington, D.C. were subsequently acquired and analyzed. The results of these measurements were in good agreement with those from tissues obtained from New York City and no statistically significant differences in plutonium concentration other than in lymph nodes were detected among the three populations.

In addition to the soft tissues previously described, various bone types including tibia, femur, ribs, and vertebrae have been obtained from eight autopsied cases from Mobile, Alabama. Analyses for plutonium concentrations in these bones have provided valuable data for describing the distribution of this nuclide at various trabecular and cortical sites in the skeleton.

O'Connor, Lee and Kneip (Laboratory for Environmental Studies) are investigating the abundance and distribution of PCBs in various planktonic organisms in the Hudson River. Samples of microzooplankton, macrozooplankton, ichthyoplankton and algae have been collected regularly over a 250 km stretch of the Hudson River from New York City to Albany. PCB analysis has been carried out, where feasible, for individual species, as well as for hatch samples of plankton.

PCBs have been detected in all of the groups of organisms examined, including amphipods (*Gammarus* spp), mysids (*Leptodora*), fish larvae (*Alosa* spp, *Morone* spp), and copepods (*Eurytemora*, *Halicyclops*). Concentrations of PCBs in these groups are rather uniform, despite the different positions of the organisms in the estuarine food chain.

The highest PCB concentrations in plankton from the Hudson occur in the vicinity of known sources, e.g., at the Troy Dam, and in the vicinity of major population centers, such as Kingston, Newburgh, and Tarrytown. Despite the presence of a major upstream source of PCBs for the estuary, distribution in the plankton does not show a decreasing, downstream gradient. Instead, there exist peaks of PCBs in the biota which are, as yet, not identified with particular point sources.

Concentrations of PCBs in the plankton, many of which are important fish food organisms, certainly contribute to the PCB body burden in fishes. Some Hudson River fish, such as striped bass, eels, and white perch maintain PCB body burdens far in excess of the 5 ppm FDA action limit. However, PCB concentrations in the plankton and fish do not

appear to be related in a manner suggestive of the operation of biomagnification in the system.

O'Connor and Lee (Laboratory for Environmental Studies) have been carrying out studies to determine the lethal and sublethal effects of PCBs on aquatic organisms, with particular reference to respiration, tissue partitioning, and uptake kinetics. In studies with amphipods (*Gammarus* spp) the effects of PCBs on respiration were determined and it was found that sublethal concentrations had a minimal but detectable effect on respiratory metabolism.

The kinetics of PCB uptake by *Gammarus*, *Neomysis* and striped bass were best described by a logarithmic function, with a k value of approximately 10^4. Steady state uptake levels were not directly determined, but were calculated from uptake curves. For all species, PCB uptake from water was concentration dependent, although the estimated steady state values were independent of concentration.

Experiments designed to determine the contribution of PCBs in the diet to total body burden in striped bass showed that: 1) cross-gut assimilation of PCBs in fish has an efficiency of 95-99%; 2) PCBs which enter fishes with food have a shorter half-life in the body than those which accumulate directly from water; and 3) tissue partitioning of PCBs accumulated via food occurs at a much lower rate than that of PCBs taken directly from water. A significant portion of the PCBs entering fish via food is first accumulated in the liver, transferred to bile, and then excreted with the feces. PCBs which enter fish directly from the water (i.e. by gill uptake) are distributed to tissues such as muscle and kidney to a greater extent than those which enter via food. Experiments in progress will determine the mechanisms of PCB transport in fish and the kinetics of PCB partitioning among tissues.

O'Connor, Kneip and Lee (Laboratory for Environmental Studies) have recently initiated a study of the transport of PCBs in coastal marine food chains. These studies utilize three-step model marine and estuarine food chains consisting of sediment, polychaete worms, and marine flatfishes (*Trinectes* and *Pseudopleuronectes*). The presence and abundance of levels of ^{14}C-labeled PCBs at each step of the chain are being used to estimate the quantities of PCBs which may be expected to occur in edible marine fishes that are exposed to PCB-contaminated marine sediments. Places where such exposure occur include New York Harbor, the New York Bight, and other coastal marine waters.

O'Connor, Kneip, Laurer, Cohen, N. and Daisey (Laboratory for Environmental Studies) have initiated a program to identify the contaminants in dredged materials from the New York metropolitan region. Samples taken from the bottoms of the Hudson River, New York Harbor, Newark Bay, the Lower Bay and other locations are being analyzed by a variety of techniques including gas, thin layer, and liquid chromatography, atomic absorption, and X-ray fluorescence to quantify major classes of

pollutants. The pollutants being studied include PCBs, PAHs, petroleum hydrocarbons, metals and others. The work will permit the identification of samples from various locations by their contaminant content. The purpose of the research is to determine whether highly contaminated sediments dumped at sea from urban areas can be effectively isolated from transport processes which would introduce the contaminants into the food chain leading to man.

O'Connor and Kneip (Laboratory for Environmental Studies) have continued their research on the distribution and abundance of cadmium (Cd) in blue crabs taken from the Hudson River. Previous work has shown that the crabs contain substantial amounts of Cd, and that most of the Cd is concentrated in the hepatopancreas. The work of the past year has focused on the factors that lead to the concentration of Cd by the hepatopancreas.

The Cd in the hepatopancreas of the blue crabs is stored in a protein-bound form. Biochemical analysis of the protein shows that it has a molecular weight of about 10,000 and that it resembles the metal-binding protein metallothionine which is found in vertebrates. The synthesis of the Cd-binding protein of crabs can be induced in the laboratory by exposure of the crab to Cd.

These data are of importance in determining the extent to which humans may be exposed to Cd from seafood, since the hepatopancreas of blue crabs is frequently eaten along with the muscle. The chemical state of Cd in the hepatopancreas, in the organic form, or as a metallo-organic complex may have a profound effect on Cd toxicity.

O'Connor and Kneip (Laboratory for Environmental Studies) are continuing studies of the health aspects of contaminants in the New York Bight. Research during 1980 has concentrated on determining the sources, sinks, and distribution of metallic and organic toxicants in the New York Bight and on relating the concentrations of toxicants observed in the aquatic biota to human body burdens.

Mercury and cadmium are the most significant metals contaminating the Bight, and at least 35 organic compounds (at least 15 PAHs and 20 chlorinated organics) have been identified in the water column, in sediments, and in the biota. The list of organics detected includes several potent carcinogens (e.g., BAP, BEP, DMBA, Dieldrin), as well as several broadly distributed ecotoxins, such as DDT (and its metabolites) and PCBs.

In general, it has been determined that organisms from the ocean adjacent to the Hudson River and to the New York Harbor contain few organic contaminants at low concentrations. Some organisms contain significant levels of mercury and cadmium. The major sinks for all contaminants are the finely divided sediments. The major source of PCBs in the system is industrial discharge, and the major sources for PAHs

are industrial and shipping operations in the Port of New York, and sewage discharges.

Initial estimates were made of possible human contamination by PCBs, the most abundant contaminants in the fishes and shellfish in the Bight region. The estimates suggest that PCB contamination of human beings is less than one percent of the lowest levels reported to cause effects in laboratory animals. Work is continuing on the estimation of the dose to human beings for other contaminants, and on the determination of the probable routes and rates of transport of metals and organic compounds in the system.

Lee (Laboratory for Environmental Studies) has continued studies on the microbial degradation of PCBs by fungi and anaerobic bacteria in nature. This research has concentrated on the isolation of fungi and anaerobic bacteria which can grow in the presence of PCBs and degrade the compounds. The organisms have been found both in clean environments and in areas contaminated by PCBs. Methods for measuring PCB degradation have been developed and fungal and anaerobic bacterial cultures have been established from a number of aquatic environments from across the United States. Studies to screen these new isolates for PCB degradation activity have begun.

Growth data from organisms derived from PCB-contaminated Hudson River sediments have shown that certain of the fungi and anaerobic bacteria can degrade ^{14}C-labeled Aroclor 1254. Radioactive carbon dioxide and products tentatively identified as chlorobenzoic acid and a pentachloro biphenolol, as well as several other unidentified ^{14}C-labeled compounds have been recovered from extracts of a culture medium treated with the labeled PCB. The presence of these products correlated with a decrease in the total PCB concentration in the culture system. Changes in the peak ratios of gas chromatograms of PCB extracts subjected to microbial activity were also observed. This may indicate selective metabolism of individual PCBs, possibly by dechlorination. Quantitative estimates of PCB utilization by the fungi and anaerobic bacteria, the identification of metabolites produced, and further characterization of the microbial cultures are continuing.

The current studies are being expanded to include studies designed to show dechlorination. In these studies, microbial systems will be given a PCB containing either 5 or 6 chlorines as a substrate. If the PCB is dechlorinated, new PCBs containing 5 or fewer chlorines will be formed, and these will be detected by gas chromatographic analysis of culture extracts.

Cohen, N. and Eisenbud (Laboratory for Environmental Studies) have continued research directed at increasing knowledge of the biogeochemical behavior of plutonium in the estuarine environment. As a part of field studies conducted to measure plutonium concentrations in water and sediment during the past year, continuous water sampling stations have

been installed near Albany, New York and in the effluent discharge canal of the Indian Point nuclear reactors. In addition, sediment core samples have been taken over a 165 mile area of the estuary extending from N.Y. Bay to Albany. In conjunction with these measurements, a series of *in vitro* experiments using ^{237}Pu were conducted to define the degree of association (i.e., partitioning) between plutonium isotopes and sediment particles in a simulated water-sediment environment. The distribution coefficient, K_d, was determined as a function of time, salinity, sediment concentration, particle size, pH, and various chemical parameters characteristic of the water phase.

In addition to studies on plutonium transport in the estuarine environment, research has continued on the fate of the fallout and/or the reactor-produced radionuclides ^{137}Cs, ^{134}Cs, ^{60}Co, ^{54}Mn, and the naturally-occurring radionuclides ^{40}K, ^{226}Ra, and ^{232}Th. These nuclides have now been measured in 500 samples of Hudson Estuary sediment, water, fish, and aquatic vegetation. Measurement techniques incorporating both NaI(Tl) and Ge(Li) detectors, coupled to computer-based multichannel analyzers, were used to identify and quantitate the above nuclides. Results to date indicate a continued equilibrium trend with time in the ^{137}Cs concentration of surficial sediments, and in the total sediment accumulation of ^{137}Cs the vicinity of the Indian Point reactors. This trend reflects the constant low-level input and removal of reactor and fallout-derived ^{137}Cs.

Cohen, N. and Hirshfield (Laboratory for Environmental Studies) have determined concentrations of gamma-emitting fission products and actinides in beach coral samples collected in 1956 from various islands that are part of the Enewetak Atoll. The majority of these samples were obtained from within nuclear weapons test site craters. One sample, from Pearl Island, containing unusually high concentrations of these nuclides, was separated into five fractions: two sizes of coral/coralline fragments and three genera of Foraminifera (*Calcarina, Amphistegina,* and *Marginopora/Sorites*).

Radionuclide concentrations in each fraction have been determined, and the implications of uptake by the exoskeleton of these biota and their possible employment as indicator organisms have been studied. The distribution and concentration of radionuclides in the 1956 samples have been compared with those collected by the AEC in 1973.

O'Connor (Laboratory for Environmental Studies) is completing simulator studies on the effects of chemical, thermal, and hydrostatic pressure on planktonic organisms entrained in steam electric-station cooling water. The studies are being carried out using a power plant condenser tube simulator that is capable of simultaneously exposing test organisms to one or more of the stresses (pressure, temperature, turbulence, biocides) which occur in a prototype condenser tube system. The results are being applied to the evaluation of entrainment impact on plankton and ichthyoplankton, and to the development of new concepts for the design of power plant cooling systems.

It has been determined that chlorination, or biocide application, is the most significant factor affecting survival of plankton during condenser tube passage. Among striped bass eggs and larvae, *Gammarus* and *Neomysis*, sublethal concentrations of chlorine applied in combination with normally sublethal levels of temperature, mechanical stress or hydrostatic pressure change cause significant mortality. Chlorine therefore may act synergistically with other stresses, whereas most other stresses act additively when applied simultaneously.

The immediately lethal effects of stress due to condenser tube passage can be readily predicted since the levels of temperature, chlorination, and flow which will result in reduced mortality among entrained organisms have been determined. Long-term sublethal effects on respiration, growth, and fecundity of various crustacea, however, may occur even at very low levels of stress. Although thermal shock and hydrostatic pressure changes appear to cause little if any long term impact, chlorination may affect individual growth in *Gammarus* and it could also reduce fecundity among exposed individuals which survive condenser passage.

Eisenbud, Kneip, Laurer and Cohen, N. (Laboratory for Environmental Studies) are continuing their studies, in collaboration with investigators in Brazil and at Stanford, of the mobilization rate of an estimated 20,000 metric ton natural deposit of thorium that has existed for about 80 million years near the summit of a hill (the Morro do Ferro) in the State of Minas Gerais, Brazil. Because the chemistries of thorium and plutonium are so similar, the deposit is being studied as an analogue of a nuclear waste repository that has been breached. The deposit is in an advanced state of weathering, and is situated in an area relatively undisturbed by human activity.

Two basic approaches are being used, on the assumption that the abundant rainfall of the area (170 cm/yr) is the primary agent of mobilization. The methods used are both prospective and retrospective.

In the prospective study, the mobilization of thorium by a stream that drains the hill is being investigated by chemical measurements of water flowing through a flume built to contain the flow and permit continuous measurements of flow, as well as continuous collection of water samples. Until now, only grab samples have been collected, but sufficient numbers of samples have now been analyzed to permit a first estimate of the annual fractional mobilization rate, which is tentatively believed to be between 10^{-7} and 10^{-8}. Considering the 25,000 year half-life of plutonium-239, essentially total *in situ* decay would be assured even at an annual mobilization rate of 10^{-6}.

The retrospective study is based on the assumption that the thorium and other elements may be collecting in a swamp about 2 km downstream from the hill. Cores, which have been taken from the swamp to a depth of 3.5 m, will be analyzed and dated in an attempt to reconstruct the past mobilization history.

An X-ray fluorescence method for the determination of thorium, uranium, and rare earths has also been developed and calibrated, and isotopic analysis of the thorium is being performed to determine if disequilibria exist.

B. SUMMARY OF TEACHING PROGRAM

Training in environmental health leading to graduate degrees is formally carried out in the Graduate School of Arts and Science, with several options. The largest number of students are in the Program in Environmental Health Sciences which is administered by the Institute. Students may also matriculate as collaborative majors in one of the several science departments at Washington Square, or in one of the basic medical science departments at the medical school. Non-degree training is also offered.

The major emphasis is on doctoral research training, and only students specializing in occupational hygiene are accepted for a terminal master's degree. Individual study programs prepare students for doctoral thesis research in an environmental health specialty. The programs are supplemented by advanced courses in basic sciences and by more general courses, to give a wide perspective of environmental health problems. Regardless of the type of matriculation chosen, thesis research is supervised by the faculty of the Institute.

During 1979-1980, the Institute had five training grants - three from the National Institutes of Health, one from NIOSH, and one from the Department of Energy. These grants provided support for 19 pre-doctoral trainees and 10 post-doctoral trainees. Various other fellowships provided support for 13 additional graduate students. Research grants and contracts provided salaries for research positions for an additional 37 graduate students.

In the academic year of 1979-1980, there were a total of 89 graduate students. Of these, 55 had master's degrees, 37 had passed their written preliminary examinations, and 24 had approved doctoral thesis outlines. Eight students graduated; 5 received the Ph.D. and 3 received the M.S. degree.

Not all of the 33 courses offered by the Institute are available in any given year. In 1979-1980, a total of 25 courses were offered for graduate credit. In addition, there was one full time, post-graduate two-week course for physicians, and a lecture course for second-year medical students.

C. ORGANIZATION OF THE INSTITUTE OF ENVIRONMENTAL MEDICINE

DIRECTOR AND DEPARTMENT CHAIRMAN: Arthur C. Upton

DEPUTY DIRECTOR AND VICE-CHAIRMAN OF THE DEPARTMENT: Roy E. Albert

ASSOCIATE DIRECTORS: Bernard Altshuler
Merril Eisenbud
Edward D. Palmes
Walter Troll
Benjamin L. Van Duuren

ASSOCIATE DIRECTOR FOR RESEARCH AND ADMINISTRATIVE SERVICES:
Stanley W. Dublin

LABORATORIES (Directors and Senior Associates):

Organic Chemistry and Carcinogenesis

B. L. Van Duuren (Director), S.C. Agarwal, S. Banerjee, S. Kline, U. Maté, A. Segal, J. Solomon

Biochemical Toxicology

W. Troll (Director), S. Belman, S. Garte, F. Mukai, R. Wiesner, R.A. Mufson

Experimental Medicine

R.E. Albert (Director), F. Burns, A. Penn, T. Rossman

Toxicology

E.D. Palmes (Director), H. Evans, A. Gunnison, R. Jaeger

Biomathematics

B. Altshuler (Director)

Biostatistics and Epidemiology

B.S. Pasternack (Director), B. Cohen, N. Dubin, N. Harley, M. Marmor, M. Schwartz, R.E. Shore,

Environmental Studies

M. Eisenbud (Director), T.J. Kneip (Deputy Director), N. Cohen (Assistant Director), J. O'Connor (Assistant Director), J.M. Daisey, H. Hirshfield, G.R. Laurer, C.C. Lee, M.E. Wrenn (L.O.A.), R. Ballad

Aerosol and Inhalation Research

M. Lippmann (Director), P.J. Lioy, R.B. Schlesinger, D. Spektor, S. Robinson, B. Cohen

Inhalation Toxicology and Carcinogenesis

R.E. Albert (Director), N.C. Dulak (Deputy Director), M. Kuschner (Associate Director), M. Lippmann (Associate Director), B. Pasternack (Associate Director), A. Sellakumar, C. Snyder

Interlaboratory Epidemiology Unit

R.E. Albert (Director), B. Pasternack (Associate Director), M. Lippmann, E.D. Palmes, R. Shore, N. Dubin, M. Marmor

Graduate Studies Steering Group

B. Altshuler, Mathematics (Chairman); F. Burns, Biology; N. Cohen, Radiation and Physical Sciences; H. Hirshfield, Biology; M. Lippmann, Engineering and Physical Sciences; R. Jaeger, Inhalation Toxicology; R. Shore, Epidemiology

Research Library

C. Singleton (Research Librarian)

D. ADVISORY COMMITTEE TO THE NIEHS CENTER PROGRAM

An Advisory Committee to the NIEHS Center Program of the Institute of Environmental Medicine has been established. The Committee will advise the Center on issues of scientific policy and direction, and aid it in facilitating collaborative relationships with other parts of New York University, and the scientific community.

The membership of the Advisory Committee is:

A. I. Kosak, Professor and Chairman
Deparment of Chemistry
New York University

Robert R. Raymo, Dean of the Graduate School of Arts and Science
Professor of English

Arthur C. Upton, Chairman
Department of Environmental Medicine

Roy E. Albert, Deputy Director
Institute of Environmental Medicine
Director of the Laboratory of Experimental Medicine

Stanley W. Dublin, Associate Director
Institute of Environmental Medicine

Marvin Kuschner, Dean
School of Medicine
State University of New York at Stony Brook

I. Bernard Weinstein, Professor of Medicine and Environmental Sciences
Columbia University

David Axelrod, Commissioner of Health
State of New York
Department of Health

James L. Whittenberger, Director
Kresge Center for Environmental Health
Harvard School of Public Health

III. RESEARCH PROJECTS

A. TOXICOLOGY

1. Personal Monitoring Devices for Gaseous Contaminants

The first passive gas sampling device which gave quantitative results predicatable on other than empirical bases was developed in this laboratory and reported in 1972. The device depended on diffusion of the gas molecules in accordance with Fick's First Law from the atmosphere being sampled through a known diffusion path to a highly efficient sorbent for that particular gas. It was, therefore, not necessary to use power to move the contaminated air through the sorbent as had been the case in the past. The original device, which was designed to sample SO_2 and water vapor, gave results which were in excellent agreement with diffusion theory, using coefficients of diffusion determined independently by others. More recently, emphasis has been placed upon the development of a sampler for the measurement of nitrogen dioxide (NO_2) and nitric oxide (NO), collectively designated as NO_x.

Since 1972, many samplers have been described by other investigators for the measurement of both inorganic and organic gases. These samplers have used columns of air of known dimensions or uniform plastic membrane disks as diffusion limiting barriers. Both types of sampler design have given excellent results in practical applications. However, the state of the art in membrane fabrication has not advanced sufficiently to permit the prediction from theory of the permeation constants for individual gases passing through specific membranes.

The samplers developed here are small, light (about ¼ oz.), inexpensive to construct, and simple to use. The NO_2 and NO_x devices are now being produced by a scientific supply house, and they are being widely used by governmental agencies, industries, and other universities. Applications of the samplers include the measurements of occupational exposures and of indoor and outdoor nitrogen dioxide air pollution. The Institute of Environmental Medicine, together with the U.S. Bureau of Mines, was recently presented with an IR-100 award for the development of the Palmes Passive NO_x Samplers by *Industrial Research and Development*. The publication designated the samplers as one of the 100 most significant new technical products of 1980.

Because of the wide interest in the samplers and their expanding use, recent efforts have focussed on several aspects of diffusion theory which directly affect the performance of the samplers. It was shown that the samplers can be modified by adding resistance to diffusional flux in the standard NO_2 sampler. Experiments including this finding have shown that both electrical and diffusional resistances in series are additive. Another factor that has been studied is pressure. Theoretically, the effect of pressure on sampling rate should be nil, but

recent experimental data suggest that there may be a significant reduction in sampling rate at extremely low pressures. This work was described in a Master's thesis by Robin Lindenboom entitled *The Effect of Atmospheric Pressure on the Collection Efficiency of a Passive Diffusion Dosimeter.*

A new project has been initiated to utilize another aspect of Fick's First Law, the uniformity of the concentration gradient within a fixed diffusion barrier, to measure both the efficiency and the capacity of various sorbents for organic vapors.

Sponsor: U.S. Bureau of Mines, Grant No. G0177042 and Contract No. J0308072

Principal Investigator: E.D. Palmes, Ph.D.

Co-Investigator: A.F. Gunnison, Ph.D.

Staff: Robin Lindenboom, M.S., Douglas Nesta, B.S. and John Mignano, B.S.

Consultant: Jerome Solomon, Ph.D.

Publications:

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Palmes, E.D. NO_2 and NO_x diffusion techniques. *Proc. of ACGIH Symposium on Dosimetry for Chemical and Physical Agents.* Cincinnati, 1980. In Press.

2. The Relationship Between Discriminitive Function and Age in Pigeons and Human Beings

A study has been carried out to compare the visual and temporal discrimination of normal human subjects and pigeons. In this study, normal adult and young volunteers were examined under computer-controlled laboratory conditions, and the data were subjected to computer analysis using programs developed by this laboratory. The apparatus used was almost identical to that used previously in similar studies with pigeons. Furthermore, the tests were conducted in a way that eliminated the confounding effects of language and ethnic background, which complicate most psychological tests with human subjects. Therefore, a comparison of the performances of the human and animal subjects can be made.

The results are interesting in that they showed that an objective index of several important nervous system functions can be obtained within a 30-minute test session. They also showed that the visual and temporal discriminations made by pigeons, children, and adults are qualitatively similar. There are quantitative differences, however. The adults were more adept at temporal discrimination than the children or the pigeons.

Sponsor: Institute of Environmental Medicine

Principal Investigator: H.L. Evans, Ph.D.

Co-Investigators: S.A. Daniel, Ph.D. and J.J. Teal, Ph.D.

Staff: A.M. Dempster, B.A.

3. Species Comparisons in the Neurotoxicity of Acrylamide

Acrylamide is a prototype of toxicants which produce chronic neuropathy, and studies of acrylamide are in progress. In cooperation with the National Institute of Environmental Health Sciences, the laboratory has developed an instrument to test the strength of mice. The device has been used to quantify both the forelimb and hindlimb grip strength of the mice, and it has been found that hindlimb strength is a more sensitive indicator of the effects of acrylamide and other toxicants.

A new system is also being developed that can document acrylamide toxicity even in advance of the reduction in grip strength. The sensitive new procedure, referred to as the "snacking" test, offers the mouse a brief opportunity to consume a non-essential food substance that is not available in the maintenance diet. The non-essential nature of "snacking" seems to account for its sensitivity to mild degrees of intoxication.

It has been shown that, in mice, the neurotoxicity of acrylamide is manifested by a characteristic postural change, which accompanies the loss of leg strength. The present studies have shown a similar response by pigeons to acrylamide. In other studies in which the "snacking" test was used on pigeons, it was found that, as with mice, the test was a much more sensitive indicator of acrylamide intoxication than weight loss or hindlimb weakness.

More advanced behavioral studies with acrylamide in birds revealed early evidence of intoxication in the complex discriminative function of the nervous system. A new computer-controlled method for titration of the threshold for time discrimination was developed, and normative data from humans and pigeons have been obtained. Pigeons are slightly less skillful in making time discriminations than are graduate students, who live in a world where estimation of the passage of time is of vital importance.

Sponsor: Institute of Environmental Medicine

Principal Investigator: H.L. Evans, Ph.D.

Co-Investigators: S.A. Daniel, Ph.D. and J.J. Teal, Ph.D.

Staff: A.M. Dempster, B.A.

4. New Behavioral Tests for Occupational Solvent Risks

Several procedures developed in this laboratory are now being applied to the characterization of the behavioral consequences of subacute inhalation of low levels of organic solvents. The solvents being studied are benzene, toluene, hexane, and trichloroethylene. The rationale for the work is the idea that behavioral methods may document effects that have previously escaped detection in the animal laboratory. It is hoped that the new techniques can be streamlined to produce screening tests for neurotoxicity.

One feature of this research program is the documentation of sleep disturbances by the examination of the diurnal pattern of carbon dioxide elimination. Previous work has demonstrated the feasibility of measuring CO_2 concentrations as low as those produced by a single mouse sleeping in a small (2 to 5 liter) inhalation chamber. It was found that CO_2 concentration changes with the daily light/dark cycle, reaching a minimum in the light portion of the day when the animals are sleeping.

The CO_2 concentration also provides an index of the metabolic and behavioral activity of animals which have been treated with stimulant or depressant drugs, and this index can be obtained while animals are enclosed in the inhalation chamber, where conventional instrumentation for behavioral tests is not practical.

Another sensitive behavioral index of intoxication is change in appetite, which has been shown to occur well in advance of observable changes in body weight. Parallel tests of appetite and food intake have been developed for mice, pigeons, and monkeys, and work is underway to set up new cages and related facilities for the housing and testing of the monkeys.

Sponsor: National Institute of Occupational Safety and Health, Grant No. OH 00973

Principal Investigator: H.L. Evans, Ph.D.

Co-Investigator: E.D. Palmes, Ph.D.

Staff: J.J. Teal, Ph.D., A.M. Dempster, B.A. and D. Taylor, Sc.B.

5. Metabolic Fate of Inhaled Metal-Sulfite Aerosols

The primary objective of this project is the characterization of the metabolism of ferric sulfite aerosols in terms of their potential for formation of S-sulfonate compounds ($RS\text{-}SO_3^-$) in the tissues of the respiratory tract, as a result of sulfitolysis of disulfide bonds. In the first stages of this investigation, members of the Laboratory for Aerosol and Inhalation Research worked on the generation and characterization of a monodisperse ferric sulfite aerosol. During this time, this laboratory was engaged in preliminary studies of the distribution of S-sulfonate metabolites in tissues of the respiratory tract, as well as in the plasma and aorta, following continuous exposure of rabbits to SO_2 for periods of 1-72 hours at concentrations of 3 and 10 ppm. Data from these experiments were to serve as a basis for comparison with later data obtained on the same tissues following exposure to ferric sulfite aerosols.

S-sulfonate metabolites were formed in the plasma of rabbits exposed to 10 ppm SO_2, indicating that SO_2 was absorbed at a significant rate into the blood stream, probably from the upper airways. Little or no free sulfite remained in the blood as it passed through the aorta, since S-sulfonate metabolites were not detected in the walls of this organ in spite of the presence there of a high concentration of reactive sites. These data indicate that sulfite absorbed into the blood stream during SO_2 inhalation is not widely distributed to tissues removed from the site of absorption. Slight, but statistically significant, S-sulfonate formation was detected in the posterior pulmonary lobes of these same rabbits.

Protein S-sulfonate formation was readily detected in tracheal tissues following exposure to 10 ppm SO_2 and, after 3 hours, an equilibrium was established between inhaled SO_2 and $RS\text{-}SO_3^-$ concentration. However, prolonged exposure to 10 ppm SO_2 (longer than 24 hours) resulted in increased $RS\text{-}SO_3^-$ formation. This was attributed to increased mucus production. Albumin, a component of mucus, is known to form $RS\text{-}SO_3^-$ on exposure to sulfite and a second component, glycoprotein, contains numberous interchain disulfide bonds which are potentially reactive sites for sulfitolysis.

The reactivity of mucus glycoprotein with sulfite was investigated further because of the possibility of an alteration in the viscoelastic properties of mucus by sulfitolysis, which might explain the decreased mucociliary clearance observed by some investigators following SO_2 exposure. Mucus glycoprotein was collected by extraction of the soft membranous tissue of the tracheal wall with 6M urea and separation of high molecular weight glycoprotein by Sepharose gel filtration. This glycoprotein reacted with $^{35}SO_3^=$ to form a covalently bound radioactive product with the same separation characteristics as the high molecular weight glycoprotein that was presumably the S-sulfonate derivative. An attempt was made to demonstrate this reaction *in vivo* by collecting

mucus from the nasal passages of SO_2-exposed rabbits and analyzing the high molecular weight glycoprotein fraction for S-sulfonate content. However, this metabolite could not be detected by conventional colorimetric analysis. Therefore, if reaction did occur, its quantitative importance was minimal compared to S-sulfonate formation in the tracheal wall as a whole.

This year, the Laboratory for Aerosol and Inhalation Research developed the capability to generate a monodisperse, SO_2-free, ferric sulfite aerosol with mass median diameter of 0.34 μm (σ_g 1.29). Rabbits were exposed to this aerosol at concentrations of approximately 0.5 mg SO_3/m^3 (SO_3 in ferric sulfite complex) for a period of one hour through a cuffed endotracheal tube. This delivery system was used, not as a model for physiological exposure, but as a way to bypass the nasal turbinates and to assure that the aerosol reached the trachea. Although this phase of the project is not complete, data accumulated to date indicate that exposed rabbits have no higher RS-SO_3^- concentrations in the lobes of their lungs or tracheal walls than do control animals.

Sponsor: Electric Power Research Institute, Contract No. RP 1157-2

Principal Investigator: A.F. Gunnison, Ph.D.

Co-Investigator: E.D. Palmes, Ph.D.

Staff: L. Dulak, Ph.D., J. Zaccardi, M.S. and T.J. Farruggella, M.S.

Publication:

Gunnison, A.F., J. Zaccardi, L. Dulak and G. Chiang. Tissue distribution of S-sulfonate metabolites following exposure to sulfur dioxide. *Environ. Res.* In Press.

6. Distribution and Fate of Inhaled Sulfur Dioxide and Ingested Sulfite

Sulfur dioxide is a commonly encountered air pollutant, and sulfite salts are present in our diet as food additives. Intake of exogenous sulfite from these sources augments a usually much larger sulfite burden which is generated endogenously from the catabolism of sulfur-containing amino acids. The chemical reactivity of sulfite and its toxicity *in vitro* attest to the potential mammalian toxicity of this substance. However, under usual circumstances, mammals are well equipped to withstand a large sulfite burden by metabolizing it to sulfate, a much less toxic chemical species. This metabolic step is catalyzed by sulfite oxidase, an enzyme with a high turnover number which is present in most mammalian tissues. The importance of this enzyme to the health of human beings is

readily apparent from several documented cases of deficiency in this enzyme in infants, resulting in severe mental and physical retardation and early death.

In previous years, this laboratory has shown that sulfite oxidase activity varies significantly among species. The rat, which has been the species of choice for most sulfite toxicity studies, has about 10 to 20 times greater sulfite oxidase activity than the human and, therefore, may not be the most appropriate species to use as a model for human toxicity. Accordingly, a sulfite oxidase-deficient rat model has been adopted for use in sulfite toxicity studies. This model has also been used to investigate the regulation of sulfite metabolism by sulfite oxidase, by measuring the rate of accumulation of certain sulfite metabolites.

Sulfite oxidase-deficient rats were produced by a tungsten (W) abetted-molybdenum (Mo) deficiency. Proper manipulation of the W:Mo intake ratio caused the exponential loss of activity (half-life of 4 days), as determined by *in vitro* assay of liver tissue. A decline of activity to 5-10% of that of the normal adult resulted in increased systemic sulfite concentration from endogenous sources. This was shown indirectly by the excretion into the urine of two sulfite metabolites which are normally not detectable, inorganic thiosulfate ($S_2O_3^=$) and S-sulfonate ($RS\text{-}SO_3^-$). Sulfite oxidase activity continued to decline to approximately 1% of the normal adult level, where a steady state was maintained. Large increases in urinary excretion of $S_2O_3^=$ and $RS\text{-}SO_3^-$ were observed, as well as substantial increases in $RS\text{-}SO_3^-$ concentrations in the plasma, aorta, and pinna. In general, the concentrations of these metabolites were inversely related to sulfite oxidase activity. The relative inability of sulfite oxidase-deficient rats to metabolize exogenous sulfite to sulfate was also demonstrated.

Sulfite oxidase-deficient rats were used to investigate the subchronic toxicity of sulfite. These animals experienced relatively high systemic sulfite concentrations without any consistent overt effect on their health. There were no perceptible changes in weight gain or maintenance, hematocrit, hemoglobin concentration, erythrocyte reduced glutathione concentration, prothrombin time, or hepatic thiamine concentration. However, similar sulfite levels in rats with normal sulfite oxidase that were fed sulfite did produce frank anemia and a slight decrease in hepatic thiamine concentration. These effects were attributable to the reactions of sulfite with dietary constituents and not to reactions occurring in the body tissues.

Studies in sulfite oxidase-deficient rats of the effect of sulfite on reproductive capacity and *in utero* morphological development revealed no statistically significant deviation from control animals in any of the parameters tested. These included mating index, resorptions, litter size and weight, and soft tissue, skeletal and external morphological malformation.

Young, female, sulfite oxidase-deficient rats (less than 5 months of age) demonstrated a low incidence of mammary adenocarcinoma (4/149) compared to age- and sex-matched controls (0/143) with normal sulfite oxidase activity. The tumors were discovered as palpable lumps in animals which had been on test between 40 and 65 days. Although this difference in incidence is not statistically significant by Fisher's exact test, the expected rare spontaneous occurrence of this tumor type in rats of this age strongly suggests that the tumors were, in fact, treatment related.

From a historical perspective, these investigations of sulfite toxicity resulted directly from studies of the sulfitolysis of disulfide bonds, first in plasma proteins and, later, in several tissues containing relatively high concentrations of elastin. Recently, these studies have focused on the identification of those specific proteins in rabbit plasma that contain sulfite-reactive disulfide bonds. It was shown that sulfite lysed the mixed disulfide bond of non-mercaptalbumin, forming S-sulfonate groups, and reacted similarly with plasma fibronectin. The former reaction was judged to have little or no physiological significance while the latter is currently under investigation. These two proteins accounted for all of the reactive disulfides in rabbit plasma protein.

Sponsor: National Institute of Environmental Health Sciences, Grant No. ES00613

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7. Pulmonary Physiological and Histological Effects of Inhaled Fe(III) - S(IV) Aerosols

One of the component classes of ambient air pollution which has been linked to possible adverse health effects is particulate sulfur oxide. It is, however, not clear which chemical species within this class may be responsible for such effects. Some of the observed irritant effects have been attributed to stable complexes of transition metals and S(IV) found in the oxidation of SO_2. S(IV) has been found in ambient air in Pasadena, CA and New York, NY and in plumes from some smelter plants and coal-fired boilers.

The effects of inhaled Fe(III)-S(IV) aerosols are being examined, using the rabbit as a model, by monitoring changes in airway resistance, the regional deposition of an inert aerosol, and the mucociliary clearance of an inert aerosol. Histological changes in the airways due to exposure are also being examined.

Rabbits are obligate nose breathers, and their nasal airways are much more efficient filters for inhaled particles than those of human beings. Exposure of rabbits to test aerosols via the nose often results in minimal penetration of inhaled particles in the tracheobronchial tree. Thus, an oral exposure device was developed which allowed direct and efficient delivery of aerosol to the lower respiratory tract. Use of this device does not require anesthesia, a distinct advantage in studies of pulmonary physiological responses to inhaled irritants.

Deposition and clearance measurements are obtained by external monitoring of radioactivity within each animal, using a pair of 2"

diameter by 3" NaI(Tl) scintillation detectors, following exposure to an aerosol of Fe_2O_3 tagged with ^{99m}Tc. Control deposition and clearance tests have been completed, and exposure to the S(IV) aerosols are beginning.

Sulfuric acid exposures are being performed to provide a positive control for the tests using the S(IV) aerosols, and to determine their responses vis a vis those seen in previous studies with humans and donkeys. The generation system for H_2SO_4 mist has been constructed and is currently being calibrated. The aerosols are produced using a DeVilbiss #40 nebulizer, the output of which passes through a temperature and humidity controlled mixing/breathing chamber prior to delivery to the rabbits.

Respiratory NH_3 has been reported to chemically neutralize inspired H_2SO_4 particles in the upper airways (Larson, *et al.*, *Science 197*:161, 1977). This reaction offers some protection against the irritant effects of inhaled H_2SO_4. A technique is being developed to continuously measure ammonia in the expired air of rabbits. The method is based on the chemiluminescent detection of NO_x which is formed by the oxidation of ammonia within the analytical system. NH_3 concentrations are measured in the exhaled air of rabbits breathing normally, and in rabbits having tracheal cannulae, to determine regional concentrations of NH_3 within the respiratory tract.

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8. Effects of Sulfuric Acid Mist on Mucociliary Clearance and Respiratory Mechanics in Human Beings and Animals

Air pollution is a complex mixture of gaseous and particulate compounds. One major class of chemical pollutants is the group of sulfur oxides. It is now widely believed, though not proven, that acidic aerosol species such as sulfuric acid may have greater pathophysiological potential than other sulfur oxides or aerosol species, such as ammonium sulfate.

Previous studies in this laboratory have shown that one-hour exposures to 0.5 μm (MMD) sulfuric acid aerosol at the current occupational threshold limit value concentration of 1000 μg/m^3 depressed bronchial mucociliary clearance in 9 of 10 healthy nonsmoking adults. In contrast, a lower sulfuric acid concentration of 100 μg/m^3 produced a significant acceleration in bronchial clearance in human subjects. In previous studies with donkeys, it was shown that chronic exposure to H_2SO_4 at 100 μg/m^3 produced persistent effects even though single exposures did not. None of these exposures produced any significant changes in measurements of respiratory mechanics or in mucus transport velocity within the trachea.

One useful experimental approach to pollution health effects is to study a sub-group of the general population which has respiratory impairment that renders the group more susceptible to pollutant exposures. Epidemiological studies suggest that exposures to ambient sulfate aerosols may lead to exacerbation of asthma.

In current studies, two human subject groups are being examined through the use of ^{99m}Tc-tagged Fe_2O_3 particles of 4.1 μm MMAD. One group contains 10 healthy nonsmokers, while the other group consists of 10 individuals with either a past medical history of asthma and/or bronchitis or current symptoms. Both groups are being exposed for one hour periods to 0 (control), 100, 300, or 1000 μg/m^3 of 0.5 μm (MMD) H_2SO_4. Because the group with asthma or bronchitis was expected to have baseline upper airway broncho-constriction, a proximal shift of the regional deposition of the test aerosol was expected. This shift should result in a deposition pattern for the 4.1 μm Fe_2O_3 similar to that observed in our first group of healthy nonsmokers who inhaled a 7.5 μm MMAD Fe_2O_3 aerosol. Thus, a comparison between the asthma/bronchitis group and the two healthy nonsmokers groups can be based on both the same aerosol size and on similar deposition patterns.

Prior to the acid exposure, each subject performed a series of tests of respiratory mechanics including measurements employing body plethysmography, forced expiratory maneuver, and nitrogen washout. They then inhaled the monodisperse γ-tagged ferric oxide aerosol. Serial measurements of the retained aerosol were made with two radiological detection systems. Mucociliary clearance in the bronchial tree was measured using a pair of collimated 12.7 D. x 5.1 cm scintillation

detectors focussed on the right mid-lung field. Simultaneous measurements of mucus transport in the trachea were made using a six-channel probe. The results for each system were expressed as tracheobronchial clearance half-time ($TB_{\frac{1}{2}}$) and tracheal mucociliary transport rate (TMTR).

Studies of nine of the subjects with histories of asthma and/or bronchitis have been completed. Their day-to-day, within-subject variability of mucociliary clearance was greater than that found previously in healthy nonsmokers. This may be related to known alterations in mucus production often seen in persons with chronic respiratory disease, but in any case, it makes interpretations of clearance measurements difficult. Based on preliminary results, this group does not appear to be more sensitive than the healthy nonsmokers in terms of mucociliary clearance. Three parameters of respiratory mechanics, $FEV_{1.0}$, MMEF, and V_{25}, were depressed by 17, 24, and 32 percent following exposures to 1000 $\mu g/m^3$ H_2SO_4. However, these results are not statistically significant for the group ($0.05 < p < 0.10$). In two subjects, all three parameters decreased by more than 35%, and were significantly different from their pre-exposure control values. In studies such as this, in which a small group of subjects are studied extensively, changes in one or two subjects, although not statistically significant, support the hypothesis that a small percentage of the general population may be more responsive to inhaled irritants and should be given special consideration. One more subject will be studied to achieve the planned group size of 10 subjects, and a more complete analysis will then be performed.

For the study of the group of healthy nonsmokers which is also currently underway, three subjects have had all four exposures (0, 100, 300, and 1000 $\mu g/m^3$). The responses of these three subjects to the H_2SO_4 exposures were similar to those seen previously in nonsmoking normals with the 7.5 μm aerosol.

Sponsor: Electric Power Research Institute, Contract No. RP-1157-3

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Publications:

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9. Acute Toxicity of Acrylonitrile

This laboratory has identified a number of factors which influence acute acrylonitrile (ACN) toxicity. A diet high in fat, whether complete in lipotropic factors (i.e., methionine, choline, and niacin) or not was found to be associated with an increased lethality (i.e., lower LD_{50}) in both male and female rats. This diet was also associated with longer hexobarbital sleeping time and elevated brain and liver glutathione content. The increased toxicity observed after oral dosing was not seen in male rats after inhalation exposure, suggesting that hepatic metabolism is a critical factor in acute toxicity. A sex difference in metabolism was suggested by the finding that females were resistant to the toxic effects of inhaled ACN.

Covalent binding of radiolabeled acrylonitrile to macromolecules was greater in rats on the high fat diet than in control rats. This is somewhat surprising since mixed function oxidase activity was diminished while glutathione content, measured as non-protein sulfhydryl content was elevated. On the basis of these findings, one might expect that these conditions would be associated with decreased toxicity and diminished binding, rather than increased acute effects. Covalent binding studies using ^{14}C-ACN, given orally to males, showed that blood contained

the highest specific radioactivity due to ^{14}C-ACN or its metabolites, while liver contained the greatest total amount of radioactivity as a function of the administered dose. Binding to trichloroacetic acid insoluble elements in the brain, while substantial, did not explain the increased sensitivity of animals on a high fat diet to ACN.

It is hypothesized that ACN is toxic because of the metabolic release of cyanide. Pretreatment of rats with a non-lethal concentration of ACN by inhalation protected against a subsequent LD_{75} administration of this agent. There was, however, no protection against the lethal effects of orally administered cyanide at the LD_{75}. In a second set of experiments, males were no more resistant than females to the effects of cyanide. Females were much more resistant than males to the acute toxic effects of inhaled, but not orally administered ACN.

To further explore the sex differences in resistance, the effects of enzyme induction by the polychlorinated biphenyl (Aroclor 1254) were studied. There appeared to be a slight increase in susceptibility to ACN in enzyme-induced males compared to control males. However, enzyme-induced females were clearly more susceptible to ACN than were control females. In fact, the susceptibility of enzyme-induced females was similar to that of control males. In the above studies, the predominant agonal signs of ACN toxicity were apparently due to increased sympathetic nervous system activity. In other experiments, animals were pretreated with the anticholinergic agent, atropine. This pretreatment eliminated many of the ACN toxic signs but caused no alteration in the lethality of ACN.

Since enzyme induction increases sensitivity to ACN toxicity, the basis for the tolerance developed to ACN is unclear. Experiments are being designed to determine if tolerance can be induced by compounds which are chemically similar to ACN but which do not contain cyanide.

Plans are also being made to directly determine the enzyme metabolizing activity for ACN; both the vial equilibration and radiolabeled ACN binding techniques will be used. Information about the metabolism of ACN is important for understanding both the compound's carcinogenic and acute toxic actions. Finally, as part of the effort to study carcinogenicity of ACN, studies have recently been begun to determine the extent of ACN binding to DNA.

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10. Biochemical Effects of Oxidant Air Pollutants

In vivo exposure of rats to ozone or nitrogen dioxide results in a dose-dependent decrease in superoxide anion radical production ($O_2^{\cdot-}$) by alveolar macrophages isolated from the exposed animals. When alveolar macrophages from ozone-exposed animals were stimulated with phorbol myristate acetate, the level of $O_2^{\cdot-}$ production ranged from 85.9% of control at 3.2 ppm-hrs ozone to 7% of control at 10.5 ppm-hrs. Similarly, $O_2^{\cdot-}$ production by PMA-stimulated macrophages from NO_2-exposed rats ranged from 78% of control at 18.3 ppm-hrs NO_2 to 14.5% of control at 51 ppm-hrs. Since the viability of the alveolar macrophages obtained from ozone or NO_2-exposed animals was 88% or better in all cases, as judged by both Trypan blue exclusion and lactate dehydrogenase release, the decreased ability of these cells to produce superoxide anion radical cannot be attributed to a pollutant effect on cell viability. This diminution in superoxide anion radical production by alveolar macrophages from the pollutant-exposed animals might account, in part, for the ability of these two air pollutants to potentiate bacterial infections in laboratory animals.

In collaboration with Dr. Walter Troll, an hypothesis has been proposed that suggests that the production of superoxide anion radical and related species by activated inflammatory cells plays a role in tumor promotion. The known inflammatory properties of tumor promoters had been considered to be incidental, in view of the lack of correlation between tumor promotion and the degree of inflammation. However, the usual measures of inflammation, erythema, induration, and infiltration of inflammatory cells, do not necessarily directly correlate with release of free radicals. In recent studies, comparison was made of the ability of the potent tumor promoter phorbol myristate acetate (PMA), as well as less active PMA analogs and nonphorbol ester tumor promoters, to stimulate $O_2^{\cdot-}$ production by human polymorphonuclear leukocytes.

The rate of $O_2^{\cdot-}$ production was found to correlate with the tumor-promoting activities of the phorbol esters, but not with their published inflammatory activities. Mezerein and teleocidin B were slightly better stimulators of $O_2^{\cdot-}$ production than PMA, and acetic acid was inactive. All-trans retinol, retinyl acetate, and retinoic acid were found to be effective inhibitors of $O_2^{\cdot-}$ production in polymorphonuclear leukocytes stimulated with the tumor promoter phorbol myristate acetate. Retinol similarly inhibited cells stimulated with mezerein or teleocidin B. No-effect concentrations of the protease inhibitor antipain potentiated the inhibitory effect of low levels of retinol. Higher concentrations of retinol and antipain resulted in an additive or less than additive

inhibitory effect. Thus, both retinoids and antiprotease compounds, which are known inhibitors of tumor promotion, also inhibit the production of active radical species by stimulated phagocytic cells.

An unexpected concomitant of the toxicity of cadmium, a relatively inert and stable metal, is the production of oxidation *in vivo*. A potential mechanism for this action is suggested by these studies since they showed that cadmium enhances the production of O_2^-., a reactive oxygen species, in digitonin-stimulated human granulocytes and rat alveolar macrophages. As previously reported, cadmium concentrations ranging from 3.6×10^{-2}M to 3.6×10^{-4}M inhibited O_2^-. production in digitonin-stimulated phagocytes. However, when the phagocytes were stimulated in the presence of 3.6×10^{-5}M cadmium, the production of O_2^-. was markedly increased to 164% to 321% of control values. Such enhancement of O_2^-. production by low level cadmium might not only provide an explanation for the oxidizing effects of cadmium observed *in vivo*, but it may also be pertinent to an understanding of the pathogenesis of some of the disease states associated with exposure to this ubiquitous pollutant.

Sponsor: National Institute of Environmental Health Sciences, Grant No. ES00673

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11. Hematological Effects of Environmental Agents

Past efforts have focused on the methodology of assaying lipid and protein fluidity in cellular membranes by fluorescence polarization. Preliminary studies using 1,6-diphenyl-1,3,5-hexatriene have demonstrated that the probe/membrane ratio is critical in the measurement of lipid fluidity. The measurement of membrane protein fluidity by polarization assay of the innate fluorescence of membrane tryptophan has not yet been completely validated. Preliminary studies do suggest that these techniques can provide interesting information concerning the effects of oxidizing agents, metals, lipid peroxide decomposition products, and perhaps other environmental toxins, on the cell membrane.

Studies of benzene toxicity have continued in collaboration with Dr. Carroll Snyder and his colleagues in the Laboratory of Inhalation Toxicology. In addition, trans,trans-muconaldehyde has been purified and initial studies of the compound have begun. It is hypothesized that this alpha, beta-diunsaturated dialdehyde is an intermediate in a minor pathway of benzene metabolism which leads to trans,trans-muconic acid. Compounds with similar structure, including those studied in this laboratory, have been found to be highly toxic. Preliminary studies indicate that trans,trans-muconaldehyde reacts with free sulfhydryl groups, inhibits the growth of human erythropoietic cell cultures, and leads to prolongation of red cell glycerol hemolysis after *in vitro* incubation. This finding is similar to that observed in mice exposed to benzene. In addition, studies by Dr. Toby Rossman indicate that the dialdehyde is toxic to growing bacteria at approximately 10^{-6}M.

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Publications:

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12. The Role of Free Oxygen Radicals in Tumor Promotion and Carcinogenesis

In polymorphonuclear leukocytes (PMNs), tumor promoters phorbol 12-myristate 13-acetate (PMA), mezerein and teleocidin, cause superoxide anion formation. However, analogues of PMA (4-Me-phorbol 12-myristate 13-acetate and phorbol 12,13-diacetate), which are inactive as tumor promoters, do not cause superoxide anion formation. Protease inhibitors, retinoids, and dexamethasone, which block skin tumor promotion by PMA, also block the formation of superoxide anion by PMNs. When retinol and antipain are used together to counteract the effect of PMA on PMNs, their blocking effects on superoxide formation are amplified. Soybean trypsin inhibitor plus retinol, and phosphoramidon plus retinol show additive effects. Thus, the action of protease inhibitors plus retinol on blocking superoxide anion formation may be due to separate mechanisms. The action of the anti-inflammatory hormone dexamethasone in this system may also be distinct, since the hormone prevents the influx of phagocytic cells. The application of several types of promotion blocking agents appears to be a promising technique for preventing cancer.

The production of superoxide anions by cell membranes is used in a variety of ways. For example, in sea urchins, the production of H_2O_2 during fertilization prevents polyspermic fertilization. Agents such as protease inhibitors and retinol which prevent the production of H_2O_2 cause polyspermy (see below). This response is similar to the action of tumor promoters in PMNs. Cocarcinogens, catechol, and pyrogallol convert oxygen to superoxide anions without cell membrane interaction. This may be the mechanism of cocarcinogenesis.

The major difference between cocarcinogenesis and tumor promotion is the timing of application. Cocarcinogens act only when applied together with the initiator, while promoters exert their action when applied at any time from the application of the initiator to one year later. While PMA is both a promoter and a powerful cocarcinogen when applied together with the initiator, cocarcinogens such as catechol or pyrogallol are not promoters. PMA causes formation of superoxide anions and free oxygen radicals by membrane perturbation, while the strict cocarcinogens act directly by activating oxygen (as does ionizing radiation). The cellular activation of oxygen may be sustained longer and

physically closer to the initiated cells, causing promotion, or this may present only one of the features of promotion. Support for the involvement of free oxygen radicals in carcinogenesis has come from the work of T. Slaga, who has shown that benzoyl-peroxide is a tumor promoter, and from studies which have shown that antioxidants inhibit tumor promotion.

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13. Sea Urchin Fertilization: A Test System for Formation of Oxygen Radicals

This laboratory has used the fertilization of sea urchin eggs as a test system for studying the effect of tumor promoters and the function of proteases and other enzymes in development. During the past year, it was shown that the sea urchin egg appears to be similar to the macrophage or the polymorphonculear leukocyte in that it can send out bursts of

superoxide and hydrogen peroxide when its cell membrane is perturbed. The first sperm contacting the sea urchin egg causes membrane perturbation, setting off a series of events that results in the excretion of measurable amounts of hydrogen peroxide into the surrounding seawater. This series of events appears to be responsible for the inactivation of the other sperm.

The sea urchin egg is also similar to the macrophage and the polymorphonuclear leukocyte in that the formation of the hydrogen peroxide is inhibited by two agents which are known to inhibit tumor promotion, the protease inhibitors and retinol. With regard to protease inhibitors, it was shown that soybean trypsin inhibitor prevented the formation of hydrogen peroxide when added to eggs, causing polyspermy. The same effect was also shown by retinol. Retinol and retinoic acid were also directly toxic to sperm when added at doses of 0.1 mg/ml in the seawater. To demonstrate polyspermy, 0.01 to 0.1 mg/ml of vitamin A was added to sea urchin eggs, the eggs were washed twice with seawater, and sperm were then added. As was the case with soybean trypsin inhibitor, this procedure prevented the formation of hydrogen peroxide by the fertilized sea urchin egg.

The toxic effect on sperm by retinol is interesting because one of the difficulties with the use of vitamin A as a chemopreventive agent is the compound's toxicity. The toxicity to sperm could be successfully overcome by adding superoxide dismutase and catalase, and it appears that the toxicity of vitamin A to sperm is due to superoxide formed by the auto-oxidation of the polyunsaturated chain of the vitamin. The toxicity of vitamin A in other tissues is also due to this superoxide formation, and it may be useful to add antioxidants with vitamin A to avoid some of the toxic effects.

Gossypol, a known toxic component of cottonseed oil (Adams and Geissman. *Chem. Rev.* 555, 1960) which is now generally removed before the oil is used, has been proposed in China for use in male birth control. It had been noted that in certain areas when cottonseed oil was used in unheated form as salad dressing, there was a significant birth depression. Further studies made it clear that it was the effect of the cottonseed oil on males which resulted in infertile sperm. At the suggestion of Drs. Sheldon Segal and Mario Burgos of the Rockefeller University, the effect of gossypol on sea urchin sperm was studied. Gossypol, which is similar to pyrogallol, has a number of hydroxyl groups around an aromatic ring. It seemed that, like pyrogallol, gossypol would take up oxygen to form superoxide and hydrogen peroxide, which would inactivate sperm.

Sea urchin sperm were treated with gossypol or with pyrogallol, and the sperm were indeed inactivated and incapable of fertilizing eggs. The toxicity of either pyrogallol or gossypol was counteracted by superoxide dismutase and catalase. Thus, the inactivation of sperm in both

man and the sea urchin by gossypol may be due to the formation of superoxide and hydrogen peroxide. It is probable, however, that the curious finding of the Chinese, that gossypol is a birth control device, may not be useful because of the toxicity of the compound.

In summary, sea urchin fertilization provides a useful system for studying the biological effects of oxygen radicals. The chemopreventive agents of carcinogenesis, retinol and the protease inhibitors, block the formation of oxygen radicals and cause polyspermy. The cocarinogen pyrogallol inactivates sperm by superoxide anion formation, and the cottonseed toxin gossypol, a possible cocarcinogen, acts in the same fashion.

Sponsor: Institute of Environmental Medicine

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Publications:

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14. Arterial Lesions in the Cockerel

Studies have been carried out on spontaneous aortic lesions and on the atherogenic response associated with exposure to chemical carcinogens. At 4 week intervals and over a span of 4-20 weeks of age, age-dependent changes were determined in cockerels in the prevalence, size, and proliferation of early spontaneous lesions. Serial sectioning at 50 μ intervals throughout the length of the abdominal aorta indicated the presence, in nearly all cases, of a single long lesion. These lesions grew slowly until 16 weeks of age and then increased in length by a factor of 2, between 16 and 20 weeks of age. Cross-sectional areas approximately

doubled between 12-20 weeks of age. Cell proliferation accounted for the majority of the observed increase in lesion size between 8 and 20 weeks of age. Similar patterns of proliferation were seen for lesion cells and for the underlying smooth muscle cells of the aortic media at each time point.

Proliferation levels were highest when animals were growing most rapidly and lowest when animals approached full size. These results suggested that proliferation of medial smooth muscle cells and of cells in spontaneous lesions may be controlled by the same mechanism. In contrast, were some of the responses of aortic lesions appearing in animals which received weekly injections of the polycyclic aromatic hydrocarbon (PAH) carcinogen, dimethylbenzanthracene (DMBA). There were marked increases in the size and proliferative capacity of lesions in DMBA treated animals, relative to controls. The lesions' cross-sectional areas increased 10 times between 12-20 weeks of age, and cell proliferation in the lesions was more than 6 times greater than that of underlying medial cells at 16 weeks of age. Lesion area distributions in 8-week old DMBA-treated animals were very similar to those of 20 week old controls. These results and others described in previous reports suggest strongly that chronic exposure to PAH carcinogens results in a marked acceleration of development of pre-existing aortic lesions. There is no evidence, however, that these agents induce the appearance of new lesions.

Ultrastructural characterization of aortic lesions has also been undertaken. Scanning electron microscopy (SEM) showed that the lesions in control animals and those in animals exposed to DMBA were similar. In both cases, there were 1 or 2 lesions per animal. They all appeared as raised areas along the luminal surface and were similar in cross-sectional appearance. Endothelial cells covering the lesions were uniform in size and shape and lay flat on the luminal surface, while those at the lateral margins of the lesions were oriented differently. Some of these marginal cells were elevated and projected into the lumen along their long axis. Others lay flatter and showed little outward projection. The distribution, size, shape and cross-sectional appearance of individual lesions could be readily detected by SEM. Changes in the size and patterns of development of these lesions could be detected more quickly and easily with this method than with conventional light microscopic histology.

A preliminary study has also been completed on the uptake of PAH's by aortas, *in vivo*. Uptake of ^{3}H-DMBA was measured in solubilized preparations of thoracic and abdominal aortas of roosters at 1, 4, 24, and 48 hours after a single IV injection. Uptake was higher in the abdominal aortas at all time points, and the highest uptake ratio was seen at 1 hour. No activity was detected in single cell isolates of thoracic or abdominal aorta. When the one hour intimal-medial preparations were treated with collagenase, elastase, and hyaluronidase, nearly all of the activity was recovered in the soluble fraction. When these preparations were extracted with $CHCl_3$-MeOH, more than 70% of the activity

was associated with the lipid phase. These results indicate that uptake of DMBA by aortic smooth muscle cells is probably mediated through the extracellular space. These data and others presented previously are consistent with a role for lipoproteins in the transfer of PAH's from the blood to the arterial wall.

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B. CHEMICAL CARCINOGENESIS

1. Application of Mass Spectrometry to Problems in Chemical Carcinogenesis

The mass spectrometry unit has employed gas chromatographic/mass spectral/computer techniques to elucidate the structure of compounds important in carcinogenesis. Research has continued in the mass spectral characterization of carcinogen-modified nucleosides and DNA bases. Electron impact (EI) and isobutane chemical ionization (CI) mass spectrometry was employed to establish the structure of o-acylated adducts formed in the interaction of the carcinogens dimethyl- and diethylcarbamyl chloride with deoxyguanosine and thymidine. The EI spectra of carcinogen-modified nucleosides were shown to have a principal fragment ion at base +N which permits identification of the modified base. The CI spectra produced a prominent protonated molecular ion, MH^+, which allows determination of the molecular weight.

The technique of in-beam chemical ionization mass spectrometry produces MH^+ for O^6-dimethylcarbamyldeoxyguanosine, even when conventional CI causes thermal decarboxylation to produce 2-amino-N^6-dimethylcarbamyl-deoxyguanosine. Various mass spectral methods were employed to establish 3-(2-carboxyethyl)cytosine (3-CEC) as a product of the interaction of the mouse-skin carcinogen β-propiolactone with calf thymus DNA. The EI and CI spectra of 3-CEC indicated pyrolysis. The EI mass spectrum of the volatile trimethylsilyl (TMS) derivative and the deuterated TMS (d_{27}) derivative was needed to conclusively establish the identity of 3-CEC.

Work has continued to develop computer programs to improve post-run processing of mass spectral data. Library software has been completed and the laboratory has recently linked up with the Cornell University probability based matching library searching system. In the near future, it should be possible to access the NIH/EPA mass spectral search system, which will provide an expanded library containing the mass spectra of over 39,000 organic compounds.

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2. Covalent Interaction of Trichloroethylene with Cellular Macromolecules

Trichloroethylene (TCE) is used extensively as an industrial organic solvent, and it has also been used as an anesthetic. TCE has been reported to cause hepatocellular carcinomas in B6C3F$_1$ mice, and the incidence of tumors was much higher in males than it was in females. However, TCE failed to induce tumors in Osborne-Mendel rats. Experiments

from this laboratory demonstrate that TCE does not cause tumors in ICR/Ha Swiss mice when administered by intragastric feeding, by repeated skin application and by subcutaneous injection.

To study the binding of TCE to cellular macromolecules, microsomes from liver, lung, stomach, and kidney of both male and female $B6C3F_1$ mice and different strains of rats were prepared and enzymatically characterized. 1,2-^{14}C-TCE was incubated at 37°C for 1 hour individually with different microsomes, an NADPH regenerating system, $MgCl_2$, and salmon sperm DNA. The results indicate that the covalent binding of TCE to liver microsomal protein and to DNA was significantly higher for the mice than for the Osborne-Mendel rats. Furthermore, the results show that the binding to macromolecules was greater in male than in female $B6C3F_1$ mice. These findings correlate with the observed carcinogenesis bioassays carried out in these animals.

In comparisons involving different strains of rats, the covalent binding of TCE to hepatic microsomal protein from Sprague Dawley rats was higher than that to protein from Osborne-Mendel and Fischer 344 rats. All extrahepatic tissues examined, including lung, stomach, and kidney of mice, metabolized TCE at rates comparable to that found in the liver. When microsomes were incubated with ^{14}C-TCE in the presence of SKF-525A or 7,8-benzoflavone, two inhibitors of mixed-function oxidase, a substantial inhibition in the binding was observed which was concentration-dependent. Reduced glutathione 1-methyl-2-mercaptoimidazole, mercaptoethanol, and urea decreased the extent of binding of TCE to protein. When hepatic microsomes isolated from $B6C3F_1$ mice and Sprague Dawley rats treated *in vivo* with phenobarbital (PB) and 3-methylcholanthrene (3-MC) were incubated with ^{14}C-TCE, the binding to protein was enhanced in mice and rats by both PB and 3-MC. The binding of TCE to DNA was increased by PB only. Addition of trichloropropene oxide, a potent inhibitor of microsomal epoxide hydrase, to the incubation system enhanced the binding of TCE to DNA, compared to the amount of TCE bound to DNA in the absence of the inhibitor.

In further investigations on the interaction of TCE with chromatin, which carries the genetic characteristics of eukaryotic cells, hepatic chromatins were prepared and morphologically and biochemically characterized. *In vitro* studies confirmed the covalent binding of TCE to chromatin constituents only after microsomal activation. The binding of TCE to chromatin protein was greater than to chromatin DNA. When chromatin protein was fractionated into histone and nonhistone chromosomal protein (NHCP), it was found that TCE interacted more with NHCP than with histone.

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3. Studies on the Carcinogenic Halogenated Hydrocarbons

1,2-dibromoethane (DBE), widely used as a pesticide and as a lead scavenger in gasoline, has induced a high incidence of stomach carcinomas in B6C3F$_1$ mice and Osborne-Mendel rats. It has been shown in this laboratory that DBE causes significant numbers of skin papillomas and lung tumors in ICR/Ha Swiss mice. Earlier work from this laboratory showed that DBE interacts covalently with liver and forestomach microsomal protein from mice and rats, as well as with exogenous DNA, but only in the presence of microsomes. More recently, reports from this laboratory have demonstrated the covalent binding to microsomal protein and DNA of ^{14}C-bromoacetaldehyde, a metabolite of DBE, and ^{14}C-bromoethanol, a potential metabolite. These two compounds interact directly with macromolecules, and both react to a greater extent than does DBE. Both ^{14}C-bromoacetaldehyde and ^{14}C-bromoethanol were synthesized here.

To determine whether DBE can interact with chromatin DNA rather than exogenous DNA and chromosomal proteins of target and non-target organs, forestomach and hepatic chromatin from male B6C3F$_1$ mice were isolated by sucrose gradient centrifugation. The chromatin was characterized by protein/DNA and RNA/DNA ratios and by DNA-dependent RNA polymerase activity. The cytoplasmic contamination of chromatin was examined by measuring the activities of succinic acid dehydrogenase, glucose-6-phosphatase, and 5'-nucleotidase or phosphodiesterase, the marker enzymes for mitochondria, microsomes and plasma membranes, respectively. The forestomach and hepatic chomatin were devoid of any cytoplasmic contamination as shown by the absence of the above enzymes. The chromatin was then incubated with ^{14}C-DBE in the presence of forestomach or liver microsomes and an NADPH regenerating system.

Results obtained from this study indicate that DBE bound covalently to the same extent to protein obtained either from microsomes or from chromatin of both forestomach and liver. In contrast it was found that DBE bound four to five times more to chromatin DNA isolated from forestomach or liver than it did to salmon sperm DNA. It appears from these results that the metabolically activated DBE is more reactive to chromatin DNA than to exogenous DNA. The binding of DBE to protein obtained either from chromatin or from microsomes isolated from liver was greater than the binding to liver chromatin DNA or to salmon sperm DNA. However, DBE interacted significantly more with forestomach chromatin DNA than with chromatin protein from that organ. Other experiments showed that the binding of DBE to microsomal and chromatin protein was higher in liver than in forestomach. When DBE-bound chromatin was resolved into histone and non-histone chromosomal protein (NHCP), it was found that there was greater DBE binding to NHCP than to histone from both forestomach and liver. Binding to liver histone and NHCP was also greater than to forestomach chromatin proteins.

1,2-dichloroethane (DCE) is used in the manufacture of various chlorinated solvents and as a fumigant, and it is widely used as a lead

scavenger in gasoline. It has been reported that DCE induced hepatocellular carcinomas, lung adenomas, and adenocarcinomas in $B6C3F_1$ mice, although it was negative in Osborne-Mendel rats. This laboratory has demonstrated that DCE causes significant numbers of lung tumors, skin papillomas, and carcinomas in ICR/Ha Swiss mice when applied repeatedly on dorsal skin. DCE is mutagenic in bacterial systems, and there is evidence that DCE interacts covalently with cellular macromolecules upon metabolic activation. The binding of DCE to liver protein from $B6C3F_1$ mice and to salmon sperm DNA was significantly higher than the binding seen in similar studies using Osborne-Mendel rats.

Work has continued to compare the differences in DCE binding to other target organs such as lung, in $B6C3F_1$ mice and in Osborne-Mendel rats, and lung microsomes from male mice and rats were prepared and characterized. The enzymatic and non-enzymatic binding of ^{14}C-DCE to DNA and lung microsomal protein were determined by incubating microsomes or heat-denatured microsomes separately with ^{14}C-DCE, an NADPH-regenerating system, Mg^{++}, and phosphate buffer, pH 7.4. It appears that the covalent binding of DCE to denatured lung microsomal protein from mice and rats and to DNA incubated in the presence of denatured lung microsomes was insignificant in comparison to the binding when incubated in the presence of native microsomes.

To investigate whether the cytosol fraction of lung has metabolic activity, the binding of ^{14}C-DCE to proteins of lung microsomes and cytosol and to DNA was examined in incubations with microsomes, cytosol or both. The binding of DCE to cytosol protein and to DNA in the presence of cytosol was not significant in comparison to the binding to microsomal protein or to DNA in the presence of microsomes. The binding of DCE to DNA incubated in the presence of either native or denatured cytosol was the same. The addition of microsomes to the incubation system containing either native or denatured cytosol, DNA and DCE, considerably enhanced the binding to DNA. These results demonstrate that cytosol does not activate DCE to bind to macromolecules. In fact, the results indicate that the covalent interaction of DCE with macromolecules is dependent on microsomal metabolism. When GSH was added to the *in vitro* incubation system, the binding to macromolecules was inhibited, a finding that is in accordance with the earlier report from this laboratory for DBE and trichloroethylene. The inhibition of binding by GSH would be expected if the activated metabolic intermediate of DCE was electrophilic. The binding of DCE to macromolecules was also NADPH dependent, and it was significantly enhanced by *in vivo* administration of phenobarbital and 3-methylcholanthrene to male $B6C3F_1$ mice. There was no change in the binding of DCE to lung microsomal protein prepared from either phenobarbital or 3-methylcholanthrene-treated animals, compared to respective control groups.

Continued investigation shows a marked difference in the binding of DCE to exogenous DNA and lung microsomal protein of $B6C3F_1$ mice and Osborne-Mendel rats. The covalent binding of DCE to protein of $B6C3F_1$

mice, a species that is susceptible to DCE-induced lung tumorigenesis, was 3 times greater than to protein from Osborne-Mendel rats, which are resistant to lung tumorigenesis by DCE. DCE bound 5 times more to DNA in the presence of mouse lung microsomes than with those from rats. It was also found that DCE bound significantly more to macromolecules when incubated with mouse lung microsomes than when incubated with mouse or rat liver microsomes. The difference between the binding to DNA in the presence of lung and liver microsomes of rats was not statistically significant. Therefore, there appears to be a correlation between microsome-mediated binding and species and organ susceptibility to DCE-induced tumorigenesis.

In earlier work, bromoacetaldehyde (BA), a metabolic intermediate of DBE, was shown to bind covalently to protein and exogenous DNA in the presence of mouse liver microsomes, without metabolic activation. To determine whether BA is an activated intermediate of DBE, experiments are underway to characterize the nucleoside adducts formed when BA reacts with DNA, and to compare these with nucleoside adducts produced when ^{14}C-DBE interacts with DNA in the presence of a microsomal activating system. Previously, adducts of BA with deoxyguanosine and deoxyadenosine had been prepared at pH 4.5 and identified as deoxy-N^2,3-ethenoguanosine and deoxy-1,N^6-ethenoadenosine. Subsequently, deoxyguanosine and deoxyadenosine reacted with BA at pH 7.0 and 9.5 failed to yield any additional major adducts. Deoxycytidine was also reacted with BA over a range of pH's, and a single major adduct was detected and purified. This adduct has been identified as deoxy-1,N^4-ethenocytidine.

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Co-Investigators: S. Banerjee, Ph.D. and S.A. Kline, Ph.D.

Publications:

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4. Detection and Trapping of Epoxides with Nucleophiles

Epoxides are alkylating agents that have been found by various investigators to be carcinogenic and mutagenic. They are formed as a result of metabolic or photochemical oxidation reactions of biologically inactive hydrocarbons. Epoxides are used extensively in the chemical and manufacturing industries, but, despite their obvious health hazards, very little work has been done on the determination and characterization of environmental epoxides.

This laboratory recently developed a highly sensitive method for the determination of epoxide alkylating agents on a microscale. The method involves the reaction of the epoxide with an excess of the potent nucleophile 4-(p-nitrobenzyl)pyridine, and the absorbance of the product can be measured and correlated with the epoxide concentration.

Most epoxides are highly reactive and may decompose too rapidly to be detected. However, these electrophiles can be detected and characterized by their addition reactions with various chromophoric nucleophiles. It was found that a solution of 4-nitrothiophenol in 1% aqueous NaOH reacted with a methanolic solution of epoxide under mild conditions to produce the pale colored adducts that could easily be purified by silica gel chromatography. In this reaction, the epoxides are opened regioselectively, with nucleophilic incorporation preferably at the less substituted carbon atom. The aromatic epoxides are quite similar in reactivity to the aliphatic epoxides, and they produce trans adducts. Chemical ionization mass spectroscopy is ideally suited for the detection of these adducts by the technique of selected ion monitoring, where an abundant ion characteristic of the compound is monitored.

These results are significant because the experimental conditions are mild enough for the nucleophilic trapping of epoxides, and the resulting yields of stable adducts are high. This method would be quite suitable for the trapping of atmospheric epoxides as well. For example, a known volume of air might be bubbled directly through a solution of excess 4-nitrothiophenol in methanol containing 1% aqueous sodium hydroxide, which was maintained at low temperature to minimize evaporation and decomposition. The reaction mixture might then be worked up and the adducts separated from other contaminants by the combined use of column, thin layer, high-pressure liquid, or gas chromatography. Such adducts could then be characterized by their chromatographic retention times, as well as by CI mass spectrometric monitoring of protonated molecular and $M + H - H_2O$ ions, and compared with synthetic adducts of suspected epoxides. This should prove to be a highly sensitive and useful method for the characterization of labile epoxides in the atmosphere or in the industrial environment. For example, a minor amount of styrene is converted to styrene oxide in the presence of peroxides when unsaturated polyester resins are processed. Thus, workers in the reinforced plastics industry are exposed directly to both styrene and styrene oxide. This method would be quite useful for the identification and quantitation of styrene oxide in the workroom atmosphere. Under suitable experimental conditions, the reagent could also be useful in metabolic studies for the trapping of epoxides, for example, in *in vitro* experiments with animal tissues or cell cultures.

Sponsors: Electric Power Research Institute, Contract No. RP-1222-1 and American Petroleum Institute

Principal Investigators: T.J. Kneip, Ph.D. and B.L. Van Duuren, Sc.D.

Co-Investigator: S.C. Agarwal, Ph.D.

Publications:

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5. Detection and Quantitation of Direct- and Indirect-Acting Alkylating Agents in Drinking Water

To date there is no method whereby indirect-acting alkylating agents (those requiring metabolic activation to become alkylating agents) which may be present in drinking water, can be quantitated and characterized. Studies have begun in this laboratory to develop a sensitive method whereby all alkylating agents present, both direct and indirect, may be quantitated and eventually characterized. The approach being taken involves the reaction of the alkylating species with an excess of a radiolabelled nucleophile and subsequent quantitation of the resultant adduct by liquid scintillation counting (LSC). To date, several ^{35}S-labelled nucleophilic reagents have been evaluated, including ^{35}S-glutathione. These have been reacted with known concentrations of a direct-acting alkylating agent such as methyl iodide and, after chromatographic separation from unreacted nucleophile, the adducts have been counted by LSC. Preliminary results show this to be an accurate assay that is sensitive to 10^{-6} mmol alkylating agent.

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Co-Investigators: B.L. Van Duuren, Sc.D., S. Banerjee, Ph.D., S. Melchionne, B.S. and D.C. Gatica, B.S.

6. Biological Activity of Haloalkene Epoxides

Studies in this laboratory and others have shown that a number of carcinogenic haloalkenes are metabolically activated to proximal carcinogens. Epoxides are likely intermediates, and a number of haloalkene-epoxides have been synthesized and characterized in this laboratory, including *cis*- and *trans*-1-chloropropene oxide (CPO), *cis*- and *trans*-1,3-dichloropropene oxide (DCPO), trichloroethylene oxide (TCEO) and perchloroethylene oxide (PCEO). These compounds have been previously

assayed for mutagenicity in the absence of metabolic activation in *S. typhimurium* TA1535 and in *E. coli* WP2, and for genotoxicity toward the DNA polymerase-deficient mutant *E. coli* pol A⁻. Biological studies have continued by assaying these compounds for their ability to transform hamster embryo cells in culture and to induce tumors in female ICr/Ha Swiss mice. In Swiss mice, the compounds were tested both by repeated skin application and by subcutaneous injection.

All six compounds were positive in the cell transformation assay. *Cis-* and *trans*-CPO and *cis-* and *trans*-DCPO were active *in vivo* both by repeated application and sc. PCEO was weakly active by skin application and inactive sc, while TCEO was inactive by both routes. All tumors were at the site of application. A summary of biological activity of the chloro epoxides is as follows: *cis-* and *trans*-CPO and *cis-* and *trans*-DCPO were mutagenic to *S. typhimurium* TA1535 and *E. coli* WP2, genotoxic to *E. coli* pol A⁻, able to transform hamster embryo cells in culture and tumorigenic to ICR/Ha Swiss mice. PCEO was inactive in *E. Coli* WP2 and marginally tumorigenic to Swiss mice. TCEO was inactive in *S. typhimurium* TA1535 and *E. coli* WP2, and non-tumorigenic to Swiss mice. *Cis-* and *trans*-DCPO exhibited the greatest activity of the six compounds in all *in vitro* tests, and this appears to reflect their greater stability in aqueous media at pH 7.4, rather than their inherent alkylating ability. The non-tumorigenicity of TCEO and the marginal activity of PCEO in Swiss mice parallel the inactivity of the parent alkenes, trichloroethylene and perchloroethylene in Swiss mice.

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Principal Investigator: B.L. Van Duuren, Sc.D.

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7. Interaction of Dimethylcarbamyl Chloride with DNA

Dimethylcarbamyl chloride (DMCC) is a direct-acting acylating agent, and investigations at this Institute have shown DMCC to be carcinogenic in mouse skin, to produce sarcomas following subcutaneous injection in mice, and to be a potent rat nasal tract carcinogen following inhalation.

A study was begun on the *in vitro* interactions of DMCC with calf thymus DNA. DMCC was first reacted with 2'-deoxyguanosine (dGuo) at 37°C and pH 7.0 - 7.5. A small amount of a blue fluorescent compound was isolated and purified from the reaction mixture by preparative paper chromatography, followed by preparative high-pressure liquid chromatography. The fluorescent compound is the O^6-acyl derivative of dGuo, 2-amino-6-dimethylcarbamyloxy-9-(2-deoxy-β-D-erythro-pentofuranosyl)purine (DMC-dGuo) and its structure was established from UV and NMR spectra, from electron impact and isobutane chemical ionization mass spectra, and also from the isobutane chemical ionization mass spectra taken by source insertion. DMC-dGuo undergoes a novel nucleophilic aromatic displacement reaction in which dimethylamine displaces the C-6 dimethylcarbamyloxy group to form the C-6 dimethylamino derivative, 2-amino-6-dimethylamino-9-(2-deoxy-β-erythro-pentofuranosyl)purine.

DMCC was reacted with calf thymus DNA at 37°C and pH 7.0 - 7.5. The DMCC-reacted DNA was then enzymatically hydrolyzed to deoxynucleosides at neutral pH, and the DMCC-dGuo adduct was identified in the hydrolysate by high-pressure liquid chromatography.

Sponsor: National Cancer Institute, Grant No. CA 24124

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8. *In Vitro* Interaction of β-Propiolactone with DNA

β-Propiolactone (BPL), a direct-acting acylating-alkylating agent, is an initiator of carcinogenesis in mouse skin and a complete carcinogen in a number of rodent species and tissues. The lactone is mutagenic in prokaryotic and eukaryotic cells and is a potent *in vitro* transforming agent in rodent and human cell lines. This laboratory has studied the *in vitro* reactions between BPL and chromatin and its components (DNA, histones, and nonhistone chromosomal proteins). Previously, two new DNA adducts were isolated and characterized, 1-(2-carboxyethyl)adenine (1-CEA) and 3-(2-carboxyethyl)thymine (3-CET), as well as the known 7-(2-carboxyethyl)guanine (7-CEG). Recently, the new adduct 3-(2-carboxyethyl)-cytosine (3-CEC) was isolated following *in vitro* reaction of BPL with calf thymus DNA.

BPL was reacted with 2'-deoxycytidine at 37°C and pH 7.0 - 7.5 to give 3-(2-carboxyethyl)-2'-deoxycytidine (3-CEdCyd) and a minor product $3,N^4$-bis(2-carboxyethyl)-2'-deoxycytidine (3-BCEdCyd). Acid hydrolysis converted 3-CEdCyd and 3-BCEdCyd to 3-(2-carboxyethyl)cytosine (3-CEC) and $3,N^4$-bis(2-carboxyethyl)cytosine (3-BCEC). 3-CEC was characterized by UV and NMR spectra and by electron impact and isobutane chemical ionization mass spectra of the parent compound and volatile trimethylsilyl (TMS) derivatives. 3-BCEC was characterized by UV spectra and by the electron impact mass spectra of a volatile TMS derivative. 3-BCEC is the first reported alkylation by BPL of an exocyclic atom of a base found in DNA. BPL was reacted with calf thymus DNA at 37°C and pH 7.0 - 7.5. Acid hydrolysis of the NPL-reacted DNA liberated 3-CEC which was isolated by preparative paper chromatography and preparative high-pressure liquid chromatography. 3-BCEC was not detected in the acid hydrolysate. 7-CEG was the major adduct detected in the acid hydrolysate of BPL-reacted DNA, and the ratios of the known adducts were determined to be: 1-CEA, 3-CEC, 7-CEG, 3-CET; 0.10 : 0.28 : 1.00 : 0.29. The types of adducts formed by *in vitro* reaction of BPL with DNA resemble those found for dimethyl sulfate and methyl methanesulfonate, two carcinogens with similar Swain and Scott substrate constants which thus react with nucleophiles by an S_n2 mechanism, as does BPL.

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Publications:

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9. N-Nitroso Derivatives of Drugs

Many N-nitroso compounds have been shown to be highly toxic, mutagenic in simple organisms, and carcinogenic in laboratory animals. A variety of drugs that are secondary or tertiary amines can interact with nitrite in the digestive tract to form N-nitroso derivatives. Whether a given drug will form a sufficient quantity of an N-nitroso derivative to express biological activity will depend on such factors as the concentration of the drug and nitrite, the pH of the organ, the presence of catalysts (such as thiocyanate), and the rate of hydrolysis and enzymatic degradation of the N-nitroso derivative to its biologically active

intermediate. Two examples of potent N-nitroso derivatives of drugs are the N-nitroso derivative of the antituberculin drug, ethambutol, and the beta-adrenergic agonist, ephedrine. When they were given to rodents, the former caused a 100 percent incidence of severe liver necrosis, and the latter nearly a 100 percent incidence of hepatomas. We have recently prepared the N-nitroso derivative of three drugs, propanolol (used in the treatment of hypertension and angina pectoris), hydrochlorothiazide (used as a diuretic and hypertensive agent), and phenylephrine (used as a nasal decongestant).

A solution of propranolol in 0.1M HCl was treated with an excess of sodium nitrite. After 18 hours, the precipitate was isolated and recrystallized from ethanol. The elemental analysis of the product showed the product had the expected formula of the N-nitroso derivative of propranolol. This material also had characteristic UV bands of an N-nitroso compound, 351(93) and 292(5,600) mμ, while the IR band at 1450 cm^{-1} (NO) was of increased intensity, compared to the absorption band at that wave length in the parent drug. Finally, the NMR spectrum in d-$CHCl_3$ showed a slight downfield shift of the gem-dimethyl group (δ 40 cps), more pronounced shifts of the 2'CH(δ 80 cps), and a most pronounced shift of the 3'CH_2(δ 110 cps), which is adjacent to the new N-nitroso group.

Hydrochlorothiazide in 25% acetic acid, when treated with excess nitrite ion at room temperature, yielded N-nitroso-hydrochlorothiazide. Its UV absorption had a characteristic triplet of a benzylic N-nitroso [442 (113), 402 (151), 388 (128) mμ] and its IR spectrum had a 1450 cm^{-1} peak, along with other new absorption bands.

The nitrosation of phenylephrine did not yield the N-nitroso compound alone. The nitrous acid generated also reacted with the phenol moiety in the phenylephrine molecule. Chromatography of the reaction mixture on Sephadex LH-20 led to three principal fractions - an early red solid fraction a middle yellow oil fraction and a late fraction which consisted of phenylephrine, along with some highly oxidized phenylephrine. The yellow oil collected from the Sephadex column was rechromatographed on a silica gel TLC plate. The middle (yellow) fraction on the plate was removed and extracted with methanol and then rechromatographed on a fresh Sephadex LH-20 column. This material absorbed in the UV at 435, 347, 335 (sh) and 281 mμ, while the parent drug only absorbed at 275 and 280 (sh) mμ. Work is continuing on the exact structure of this oil and the other nitrosation products of phenylephrine.

Work has been started to determine the mutagenicity of the three drugs and their N-nitroso derivatives. Based on the structure of the N-nitroso derivatives, it is likely that they will not be direct-acting mutagens. Hence, in assays with both the *S. typhimurium* (TA 100 and TA 1535) and *E. coli* strains (WP2 and WP2 uvrA), arlochlor-induced rat hepatic microsomes will be present. To date, when tested with the *E. coli* strain WP2uvrA, only the N-nitroso derivative of hydrochlorothiazide showed some weak mutagenic activity.

Sponsor: Institute of Environmental Medicine

Principal Investigator: B.M. Goldschmidt, Ph.D.

10. The Relationship Between the Chemical Reactivity and the Inhalation Carcinogenic Potency of Direct-Acting Chemical Agents

Studies carried out in this laboratory with a number of direct-acting chemical agents have suggested that the inhalation carcinogenic potencies of the compounds are directly related to their chemical reactivities.

Groups of male, Sprague Dawley rats have been exposed by inhalation to one of the direct-acting chemical agents bis(chloromethyl)ether (BCME), dimethylcarbamoyl chloride (DMCC), and epichlorohydrin (ECH) at levels of 0.1, 1.0, and 100 ppm, respectively. These agents differ substantially in their chemical reactivity, with the hydrolysis half-times being 0.5, 6, and 1000 minutes for BCME, DMCC, and ECH, respectively. The exposures were carried out for 6 hours per day, 5 days per week, for a total of 30 exposures, and the exposed animals, along with appropriate controls, received lifetime followup observation for evidence of neoplastic development.

The data showed that the probability of animals dying with respiratory tract tumors approximated 35% for all of the exposed groups, and within each group, the first tumors were observed after 350 days following the first exposure. Therefore, carcinogenicity correlates strongly with reactivity in the case of these compounds. To further test the generality of this relationship, additional compounds are being examined, both by inhalation exposure of Sprague Dawley rats, and by *in vitro* testing procedures.

The series of compounds being studied includes dichloroacetyl chloride, ethyl chloroformate, β-propiolactone, methyl methane sulfonate, and propylene oxide, with hydrolysis half-times in neutral solution of 0.22 sec, 0.30 hr, 1 hr, 9.5 hrs, and 87 hrs, respectively. In the *in vivo* studies, groups of rats will receive 30 6-hour inhalation exposures to one of the agents, with followup lifetime observation for possible tumor development. The exposure level of each agent will be based upon its chemical reactivity relative to that of BCME.

This study is now in its early stages and exposures have been completed with β-propiolactone, at levels of 5, 10, and 20 ppm. To date no tumors have been observed, although none would be expected at this early time. Corresponding *in vitro* tests are also in progress, using the V79 hamster cell mutagenesis system, with ouabain resistance as a marker, and the C3H10T½ mouse cell transformation assay system.

Sponsor: National Institute of Environmental Health Sciences, Grant No. ES 02249

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Publications:

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11. Inhalation Studies with Combined Formaldehyde and Hydrogen Chloride Vapors

a. Animal Inhalation Exposures

Preliminary experiments showed a significant carcinogenic effect in rats exposed to an atmosphere of formaldehyde (HCHO) and hydrogen chloride (HCl). In order to determine whether combined exposures to HCHO and HCl were required to produce nasal tumors, or whether HCHO or HCl alone was carcinogenic, a series of experiments was begun. These experiments involve 600 animals divided into six groups of 100. Group 1 are receiving 15 ppm of HCHO and 11 ppm of HCl (premixed), Group 2 are receiving similar levels but not premixed, Group 3 are receiving 15 ppm HCHO, Group 4 are receiving 11 ppm HCl, and Groups 5 and 6 are being sham air exposed and untreated controls. All exposures are for 6 hours per day, five days per week for the lives of the animals.

A one cubic meter hood, adjacent to the exposure chambers, is used to contain the complete generation system. Formaldehyde vapor is produced by passing air over a heated slurry of paraformaldehyde, and the effluent air stream is then directed to the mixing vessel, or directly to the inhalation chamber. The concentration of HCHO is controlled by varying the temperature of the flask and the rate of air flow over the slurry. Hydrogen chloride gas is provided by feeding a known amount directly into the chamber from a compressed gas cylinder, and exposure chamber levels of HCHO and HCl are measured at half-hour intervals.

These experiments are still in progress, and at the end of the 40th week, animals in all groups have shown a gradual increase in body weight. The rate of survival has been excellent. The highest mortality has occurred in the groups receiving the premixed combined exposures (16%), and in the remaining groups the mortality is less than 5%. Complete histopathology will be performed on all test and control animals.

b. Determination of BCME in HCl and Formaldehyde Atmospheres

It has been shown that bis(chloromethyl)ether (BCME) can be formed in atmospheres of hydrogen chloride mixed with formaldehyde. These chemicals are ubiquitous in most laboratory settings and the possibilities of exposure are great. The present studies will attempt to quantify the amount of BCME formed in known, tumorigenic atmospheres of hydrogen chloride and formaldehyde.

The mass spectrometry unit has been evaluating the technique of sorbent trapping for determining the amount of BCME present in synthetic atmospheres of hydrogen chloride and formaldehyde. Sorbent traps have been shown to be superior to cryogenic traps and solvent impingers for concentrating and releasing hazardous vapors. In addition, several sorbents do not collect water vapor and are freer of artifact formation. Preliminary experiments are being conducted to determine gas chromato-

graphic (GC) conditions, limits of detection, collection efficiency, breakthrough volume, and recovery with Chromosorb 101. - Once concentrated, the materials are thermally desorbed at the head of the GC column for GC quantitation and structural confirmation by mass spectrometric analysis. This method should be suitable for analysis of other compounds that may be formed in hydrogen chloride and formaldehyde mixtures that could, in part, be responsible for the observed tumors.

c. Detection of DNA Interaction with Bis(chloromethyl)ether, Formaldehyde, and Hydrogen Chloride Plus Formaldehyde in Bacteria, Epidermal Cells in Vitro, and Nasal Tissue in Rats by Assays for DNA Damage with S-1 Nuclease

Since the carcinogenicity of atmospheres of hydrogen chloride and formaldehyde may be due to a number of factors including the formation of strong alkylating agents, the assay for single strand DNA breakage may be a useful indicator of which agent or combinations of agents is potentially carcinogenic, without the need for isolating the offending compound(s). The current studies investigate DNA damage in cultured mouse epidermal cells, rat nasal epidermal cells, and bacteria as measured by the S-1 nuclease assay.

Successful attempts have been made to develop methods for tissue culture of rat nasal cells. These were obtained from the upper one-third of the rat nasal passages, anterior to the olfactory organs. The explants were cut into 1 square millimeter sections and cultured. The procedure, however, produced extensive bacterial contaminations. It was found that contamination could be prevented by washing the nasal passages *in situ* with an antibiotic solution containing tetracycline, penicillin, streptomycin, oxicillin, gentamicin, and fungizone in Hank's balanced salt solution. Explants were then incubated three hours and washed three more times with the antibiotic solution. The explants which showed beating cilia were grown in each of the following media plus 15% fetal calf serum and the antibiotic mixture: 1) Eagle's MEM, 2) nutrient mixture F-12, 3) MEM-D-Valine, 4) MEM-D-Valine-conditioned with used medium from culture of 3T3 fibroblasts. Fibroblast growth was extensive in cultures 1 and 2. Culture 4 showed little growth of epithelial or fibroblast cells. Culture 3 allowed epithelial growth but markedly inhibited growth of fibroblasts. The epithelial cells in medium 3 exhibited beating cilia and mucus secretion. After several days, however, fibroblasts appeared and continued to grow. It was also found that epithelial cells grow slowly in uncoated plates but faster on plates coated with histone/protamine.

These studies indicate that it will be possible to prepare nasal epithelial cells free from fibroblasts by the Selective Plating Method. This will involve the growth of explants on uncoated plates in MEM-D-Valine until ciliated outgrowths appear. Areas visibly free of fibroblasts will be cloned on uncoated plates. Incubation for 8 hours will allow fibroblasts, but not epithelial cells, to attach to the plates.

The media containing free epithelial cells will be transferred to uncoated plates and cultured in MEM-D-Valine. The procedure will be repeated if fibroblasts appear and the fibroblast-free cultures will be finally grown on histone/protamine coated plates.

The effects of BCME, formaldehyde, HCl, and HCl plus formaldehyde on DNA damage in these cells will be measured.

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Publication:

Sellakumar, A.R., R.E. Albert, G.M. Rusch, G.V. Katz, N. Nelson and M. Kuschner. Inhalation carcinogenicity of formaldehyde and hydrogen chloride in rats. Abstract #424, *Proc. Amer. Assoc. Cancer Res. 21*:106, 1980.

12. Hematopoietic Effects of Inhaled Benzene

The effects of inhaled benzene have been under investigation in this laboratory for the past six years. The purpose of the initial studies was to evaluate the peripheral blood cell responses of animals during lifetime exposures to either 300 or 100 ppm benzene vapor. Three mouse strains (AKR, C57BL, and CD-1) were chosen to study the effects of lifetime benzene exposure on differing spontaneous, hematopoietic tumor incidences. Sprague Dawley rats were chosen because of our experience with their spontaneous tumor incidences and as a species comparison with the mice.

All three mouse strains responded to the 300 ppm exposures with anemia, lymphocytopenia, and granulocytosis, while the rats exhibited only lymphocytopenia. Only AKR mice and the rats were exposed to 100 ppm, and in both species, a dose-response effect was observed in peripheral blood counts.

The predominant finding at autopsy was bone marrow hypoplasia, which was particularly severe in AKR mice exposed to 300 ppm. An excep-

tion was the study with the C57BL strain in which almost half of the exposed mice died with marrow and splenic hyperplasia. In four cases, hyperplasia was limited to granulopoietic elements. This may be important since benzene exposure of humans is linked predominantly to granulocytic (myeloid) leukemia.

Evidence for the carcinogenicity of inhaled benzene was observed by the production of three cases of myeloid leukemia and six cases of thymic lymphoma. Myeloid leukemia was observed in two CD-1 mice and in one Sprague Dawley rat. These lesions were morphologically similar to human myeloid leukemia. Although the number of cases of leukemia is small, there appears to be no known spontaneous incidence of the disease in either CD-1 mice or Sprague Dawley rats. Thymic lymphoma was observed in 6 of 40 exposed C57BL mice and in 0 of 40 controls. Spontaneous thymic lymphoma is rare in these mice but has routinely been produced with x-irradiation and/or chemical carcinogens. This may be the first production of lymphoreticular tumors in animals following the inhalation of benzene.

Attempts are underway to increase the incidence of hematopoietic tumors in animals exposed to benzene. One approach is to expose Sprague Dawley rats, and CD-1 and C57BL mice to various exposure regimens which are designed to maximize dose and minimize mortality. A short-term, high intensity exposure consisting of 1200 ppm for 6 hours per day, 5 days per week, for 50 exposures has been completed. During this exposure, the mice exhibited peripheral blood lymphocytopenia and anemia, while the rats exhibited only anemia. Following cessation of exposure, red cell counts of CD-1 mice, lymphocyte counts of the C57BL mice, and lymphocyte counts of the rats returned to control values. Animals are being held for life, and monthly blood counts will be taken. A second study with benzene involves the treatment of groups of the above animals to intermittent chronic exposures to benzene vapor, at 900, 300, or 0 ppm for 6 hours per day, 5 days per week for one week in three. These exposure regimens deliver the same total dose as chronic weekly exposures to 300 and 100 ppm. Since data for these latter exposure regimens were acquired in previous studies, comparison between the effects of these dose-rates may be made. To date, seven weeks of exposure have been completed and the CD-1 mice have exhibited peripheral blood lymphocytopenia, anemia, and neutropenia, while the C57BL mice and rats have exhibited only lymphocytopenia. Animals will be exposed, and the peripheral blood counts will be monitored for life.

Because hematopoietic stem cell dyscrasias have been linked to most forms of hematopoietic neoplasms, an investigation was undertaken to assess the effect of various benzene regimens on two types of hematopoietic stem cells. Male CD-1 mice were exposed to mean benzene concentrations of 1.1, 9.9, 103, 306, 1276, 2416, and 4682 ppm for 6 hours per day, for 5 days (Experiment 1); 9.6 ppm for 6 hours per day, 5 days per week, for 50 days (Experiment 2); or 302 ppm for 6 hours per day, 5 days per week, for 26 weeks (Experiment 3). Femoral marrow and spleen cells from

control or test animals were assayed for CFUs, the pluripotent stem cell, by the spleen colony method and for CFUc, the granulocyte, macrophage progenitor cell, by an *in vitro* agar technique.

In Experiment 1, femoral and splenic cellularities were significantly reduced at concentrations of 103 ppm or greater. Marrow concentration of CFUc was equivalent to or greater than control values at all levels. However, splenic CFUc concentration was decreased at 103 ppm and above. Femoral and splenic CFUs and CFUc per organ were depressed at 103 ppm and above. No change in colony/cluster ratio was observed. In Experiment 2, no detectable changes were observed in bone marrow. However, splenic cellularity and the number and concentration of splenic CFUs were elevated compared to matched controls. In Experiment 3, a marked depression was observed in marrow and spleen cellularity. The concentration and number of marrow and spleen CFUs and marrow CFUc were also depressed. The number of splenic CFUc were also reduced, but splenic CFUc concentration was increased relative to control. Splenic colony/cluster ratio was significantly reduced in this experiment.

These data demonstrate that 5 day inhalation exposure to benzene concentrations ten times the current TLV depresses marrow and splenic CFUs and CFUc, with splenic cells showing greater sensitivity. Stem cell depletion seems, therefore, to be involved in the pathogenesis of benzene-induced hematotoxicity.

A study evaluating the effects of ingested ethanol in combination with inhaled benzene has been expanded to include effects on marrow and on nucleated splenic cells. Inhalation exposures were made with 300 ppm benzene or air for 6 hours per day, 5 days per week for 13 weeks. These exposures were combined with either 0, 5, or 15% ethanol in the drinking water, 4 days per week *ad libitum*.

The combination of benzene inhalation and ethanol ingestion produced greater depressions of peripheral erythrocytes and lymphocytes than inhalation of benzene alone. Furthermore, the combined treatment produced a large number of normoblasts in the peripheral blood. The combined treatment also produced a greater hematotoxic response in the marrow and spleen than did the inhalation of benzene alone. Greater depressions in femoral and splenic lymphocytes and marrow granulocytes were also observed in groups undergoing combined treatment. In addition, a significant, rapid increase in splenic normoblasts was found in the peripheral blood of benzene/ethanol treated animals. These experiments also showed the appearance of atypical blood cell morphology in the peripheral blood, bone marrow, and spleen in test mice, which was present to a greater degree in mice treated with benzene and ethanol than in mice exposed to benzene alone.

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13. Respiratory Carcinogenesis: Effect of UV-1084 on Sprague Dawley Rats Using the Intrabronchial Pellet Implant Technique

This investigation was designed to evaluate the carcinogenic effect of the nickel compound 2'2-thiobis-(4-t-octylphenyl-ato)-n-propylamine

nickel II (UV-1084), in cholesterol base, administered by a pellet implant technique. This pellet technique was devised to expose a restricted portion of the bronchial mucosa to a known concentration of test material for long periods.

The average weight of the mixture of UV-1084 plus cholesterol in each test pellet was 5.18 ± 0.54 mg. The average weight of the cholesterol in each control pellet was 5.03 ± 0.60 mg.

Two hundred male Sprague Dawley rats were divided into two groups. Group I received the UV-1084 pellets, and Group II received the cholesterol pellets. Both groups showed a gradual increase in body weight up to 72 weeks and a gradual decrease in weight thereafter. The rate of survival was excellent for both groups; the first year's loss was only 5% in the treated group and 8% in the cholesterol group. The experiment was terminated at the 115th week.

No significant abnormalities were seen in the respiratory tract of either the test or the control animals. The cut surface of the lung revealed that the mid-portion of the primary bronchus of the left lobe was the most frequently affected site. This was expected since the pellet was located in this area. The bronchus was dilated, and the pellet was fixed by fibrous tissue.

Lesions were found primarily at the implantation site, and the histopathological changes in the treated group were very similar to those of the controls. Ninety percent of the animals showed infective bronchiectasis, with fibrosis of the peribronchiolar tissue. None of the test animals or controls developed preneoplastic or neoplastic changes in any segment of the respiratory tract. The results suggest that the compound UV-1084, at the given dose level, is not a tumorigen in the respiratory tract.

Sponsor: American Cyanamid Company

Principal Investigators: R.E. Albert, M.D. and A.R. Sellakumar, D.V.M.

14. Mechanisms of Mutagenesis in Mammalian Cells

In *E. coli*, *Salmonella* and many other bacteria, an inducible, error-prone system (the SOS system) is required for mutagenesis by UV radiation and by many chemical carcinogens. Bacteria which lack the genes coding for this system (*recA*, *lexA* and *umuC*) are unable to mutate after treatment with these agents. Since the error-prone system is inducible, inhibitors of *de novo* protein synthesis, such as chloramphenicol, inhibit mutagenesis by these agents.

Experiments were designed to test whether UV-mutagenesis in animal cells also requires an inducible, error-prone DNA repair system similar to the SOS system. If an inducible repair system is required, then inhibition of *de novo* protein synthesis at either the transcription or the translation level should lower the UV-induced mutation frequency. To investigate this possibility, Chinese hamster (V79) cells were exposed to either DRB (5,6-dichloro-1-β-D-ribofuranosylbenzimidazole, a transcription inhibitor), cycloheximide, or puromycin for various times (3-6 hours) following UV-irradiation (2-8 J/m^2). It was found that post-irradiation treatment with DRB resulted in a reproducible enhancement of UV-induced mutagenesis, but post-irradiation treatment with either cycloheximide or puromycin resulted in a decrease. Thus, the frequency of UV-mutagenesis does not appear to be dependent upon an inducible error-prone DNA repair pathway, since all three agents inhibit *de novo* protein synthesis.

This paradoxical effect of inhibitors of *de novo* protein synthesis was examined further by studying the effects of these inhibitors on other cellular functions. It was found that DRB stimulated DNA synthesis in both irradiated and unirradiated cells. On the other hand, cycloheximide and puromycin caused an immediate inhibition of DNA synthesis in both irradiated and unirradiated cells. The enhancement of the UV-mutation frequency by DRB may be due to stimulation of DNA replication on a dimer-containing template, causing mutations by misreplication, rather than by misrepair. Cycloheximide and puromycin inhibit DNA synthesis and may, therefore, allow more time for constitutive error-free DNA repair to occur, assuming that cycloheximide and puromycin would not inhibit repair replication.

Therefore, it appears that UV-mutagenesis in Chinese hamster cells is a function of post-irradiation DNA synthesis and is independent of post-irradiation mRNA or protein synthesis. These findings support the hypothesis that if an error-prone repair or recovery system exists in mammalian cells, such a system might be constitutive rather than inducible.

Sponsor: Institute of Environmental Medicine

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Publication:

Stone, D.S. and T.G. Rossman. Effects of inhibitors of *de novo* protein synthesis on UV-mutagenesis in Chinese hamster cells: Evidence against mutagenesis via inducible, error-prone DNA repair. *Mutation Res.* 1981. In Press.

15. Comutagenic Agents

A number of agents have been shown to increase the carcinogenicity of carcinogens. One possible mechanism of action for such "cocarcinogens" is the inhibition of DNA repair. Such agents would not be expected to act as mutagens, but they should enhance the mutagenicity of mutagens which damage DNA. Methods have been developed to screen for such agents and several comutagens have been found.

Bisulfite (sulfur dioxide) is one such agent. Under moderate exposure conditions, where survival is high, it has been found that bisulfite alone is not mutagenic in either eukaryotic (Chinese hamster V79) or prokaryotic (*Escherichia coli*) cells. However, bisulfite does act as a comutagen with ultraviolet irradiation. Bisulfite approximately doubles the mutation frequency in UV-irradiated Chinese hamster V79 cells, and it causes a greater than eight-fold increase in TrP^+ revertants in UV-irradiated *E. coli*. The comutagenic effect occurs whether cells are exposed to bisulfite during or immediately following UV-irradiation. Kinetic studies of the comutagenic effect of bisulfite in *E. coli* show that the effect decays in a biphasic manner, with a rapid component having an apparent half-life of 15 mintues and a slower component persisting for up to 120 minutes after UV-irradiation.

Experiments with several strains of *E. coli* having varying DNA repair capacities indicate that excision repair is necessary for a comutagenic effect by bisulfite. *In vitro* studies have shown that bisulfite (at optimal *in vivo* comutagenic concentrations) inhibits the activity of *E. coli* DNA polymerase I. The effects of bisulfite on the exonuclease activities of polymerase I are being investigated.

In addition to bisulfite, two other agents were found to enhance UV-mutagenesis in *E. coli*. Results with arsenite are discussed in the section on Genetic Toxicology of Metals. Ascorbic acid (vitamin C), at concentrations of 20-100 µg/ml in the plating medium, also acts as a comutagen with UV radiation.

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Publications:

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16. The Effect of l-Ethionine on "SOS Functions" in the *tif-1* Mutant of *E. Coli*

The fact that physical and chemical agents in the environment damage DNA has become a matter of great concern. This is because the failure to correctly repair such damage to DNA is correlated with increased mutagenesis in bacteria and with increased carcinogenesis in higher organisms.

In wild type *E. coli*, agents which damage DNA (UV, mutagens, carcinogens, etc.) elicit the simultaneous expression of a number of functions. These include the induction of λ prophage, the inhibition of cell division with resultant filamentation, and the induction of an error-prone DNA repair activity which is responsible for mutagenesis and production of protein X, the *recA* gene product. These activities are called "SOS functions", and the expression of these activities is thought to be under the control of a common regulatory pathway which requires functioning *recA* and *lexA* genes.

At high temperature (42°C), the *tif-1* mutant in the *recA* gene causes the expression of all of the SOS functions without detectable DNA damage or perturbations in DNA replication. Earlier studies in this laboratory have shown, however, that ethionine blocks the induction of λ prophage at 42°C and causes filamentation in a *tif sfi* derivative. This is interesting because ethionine is an ethyl analog of methionine that is a liver carcinogen, yet it does not bind to DNA and it is not mutagenic in the Ames test. To learn more about the ethionine effect, recent studies have focused on the effect of ethionine on the expression of error-prone DNA repair in the *tif-1* mutant.

Elevation of the temperature of cultures to 42°C following exposure to a very low dose of UV irradiation results in an increase in the UV-induced mutation yield in the *tif-1* strain $WP_{44S}NF$. Thermal enhancement of UV mutability in this strain was either prevented or promoted by agents known to exert parallel effects on thermal induction of λ prophage in *tif* lysogens.

$WP_{44S}NF$ bacteria were irradiated with a very low dose of UV radiation (survival was close to 100%) and plated on SEM agar with or without 10 mM ethionine. The number of Trp^+ mutants/10^7 bacteria plated increased three-fold when the plates were incubated at 42°C, compared to the yield when incubated at 30°C. In the presence of ethionine, however, the number of Trp^+ mutants/10^7 bacteria was the same as that observed when the plates were incubated at 30°C, and there was no difference in the number of revertants with or without ethionine at 30°C.

High spontaneous mutation rates are observed in *tif* strains at 42°C, and the frequency of Trp^+ revertants is higher at that temperature than at 30°C. In the presence of ethionine, the frequency of revertants is 77% less than in its absence. The effect of ethionine is not due to

nonspecific effects on cellular metabolism, since ethionine does not appreciably inhibit the incorporation of radioactive precursors into protein, DNA, or RNA.

The mechanism of ethionine carcinogenesis is not clear, although the compound is known to inhibit the enzymatic methylation of DNA and RNA in a number of biological systems. Holliday has suggested that the carcinogenic activity of ethionine may be due to the loss of methylation of specic DNA sequences, which results in changes in gene expression. Therefore, effects seen in the *tif-1* mutant may be due to changes in the methylation of the DNA. The elucidation of the effect of ethionine on *tif*-mediated SOS functions may reveal the mechanism by which ethionine acts as a carcinogen.

Sponsor: Institute of Environmental Medicine

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Publication:

Wiesner, R., E. Epstein and T. Rossman. Ethionine blocks "SOS" functions in the *tif-1* mutant. Abstract #122, *Proc. Amer. Assoc. Cancer Res. 21*:31, 1980.

17. Genetic Toxicology of Metals

Among the agents known to contribute to human cancer are a number of metals and metal-like elements, such as chromium, arsenic, nickel, cadmium and, probably, beryllium. Unlike organic carcinogens, most metals, with the exception of chromate, are not mutagenic in standard microbial assay systems. This lack of mutagenicity may reflect technical problems in the systems used to measure mutagenesis, since some of the metals are highly toxic. Alternatively, it may be that the carcinogenic metals act by a different mechanism.

To study this problem further, an investigation of the mechanism of chromate mutagenesis in *E. coli* was undertaken to determine the role, if any, of inducible error-prone DNA repair processes (SOS system). Assays were performed for Trp^+ revertants, using the fluctuation test. In this procedure, multiple samples of bacteria undergo many rounds of cell division in the presence of a non-toxic level of the metal. If a mutation (to Trp^+) occurs, a pH indicator changes color in that sample. This method demonstrated that 10 μm chromate was mutagenic to all strains of

E. coli tested, including those carrying the *recA* or *lexA* (SOS repair deficient) mutations. At higher concentrations, chromate caused the induction of lambda prophage. These results suggest that although chromate is an inducer of the SOS system in *E. coli*, its mutagenicity is not dependent upon that system and probably occurs via direct misreplication of DNA.

In contrast to chromate, arsenite was not mutagenic in either *E. coli* or Chinese hamster cells. Recent epidemiological evidence shows that arsenite may act as a cocarcinogen rather than as a primary carcinogen. Previous work from this laboratory demonstrated that arsenite can interfere with the repair of UV-induced lesions in *E. coli*. However, at the highest tolerated dose (1 mM), arsenite appeared to primarily inhibit the mutagenic SOS repair pathway. Recently, it was found that exposure of *E. coli* to lower concentrations of arsenite caused an enhancement of UV-induced mutagenesis. This enhancement occurred only in excision-proficient strains of *E. coli*. The absence of mutation enhancement by arsenite in strains lacking or defective in excision repair indicates that arsenite may interfere with the process. The finding that a *PolA* strain exhibited little or no arsenite comutagenesis suggests that arsenite does not inhibit the incision step of excision repair. The effects of arsenite on other steps of the excision repair pathway (i.e., exonuclease, polymerase or ligase activity) are being examined.

The absence of mutagenicity by arsenite, together with its activity as a comutagen, suggests that arsenite is a cocarcinogen rather than a primary carcinogen. Experiments now in progress are designed to determine whether arsenite comutagenicity also occurs in animal cells.

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Publications:

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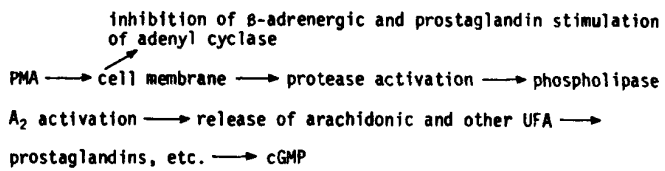
18. The Role Of Cyclic Nucleotides in Tumor Promotion

This research program involves the clarification of biochemical events affected by promoters and promotion inhibitors. Last year's Annual Report described a working hypothesis which depicted the relationship between phorbol myristate acetate (PMA)-membrane interactions, cyclic nucleotides, and other PMA-mediated events. This hypothesis, shown below, is based on the effects of PMA and promotion inhibitors in mouse epidermis and other cells.



Support for this hypothesis has recently been obtained in macrophages (Chang, *et al.*, *Proc. Natl. Acad. Sci. 77*:4736, 1980), where proteases activated the release of arachidonic acid and PGE_2. Protease inhibitors were shown to inhibit this release. PMA, which stimulates protease activity in these cells, increased PGE_2 synthesis, and this was inhibited by protease inhibitors.

The scheme of Hirata and Axelrod (*Science 209*:1082, 1980) for transmission of biochemical signals through membranes includes features of the above hypothesis and indicates that PMA may act by activation and inhibition of hormonal signals.

Mouse epidermal phospholipase A_2, which is a key enzyme in PMA-stimulated events, has been characterized. The substrate was [^{3}H] arachidonic acid-labelled phospholipids from 3T3 cells. The enzyme is particulate and requires calcium, and its activity is enhanced by detergents. The optimum conditions were found to be pH 7-9, 12 mM calcium and 0.1% taurodeoxycholate. The effect of detergents was in the order taurodeoxycholate > deoxycholate >> Triton X-100. Activity was linear

for 3 hrs at 37°C, and was a function of enzyme concentration. All groups of phospholipids were hydrolyzed with slight specificities in the order of phosphotidylserine > phosphatidylethanolamine > phosphatidylcholine > phosphatidylinositol.

PMA had no direct effect when added to the *in vitro* assay system, which suggests that it may stimulate phospholipase A_2 indirectly, perhaps by protease activation, calcium influx, or membrane alteration.

The release of unsaturated fatty acids (UFA), such as arachidonic acid, which are metabolized to prostaglandins, involves the formation of malondialdehyde (MDA). MDA has been reported to be an initiator in mouse skin (Shamberger, *et al., J. Natl. Cancer Inst. 53*:1771, 1974).

It was found that PMA stimulates an increase in mouse epidermal MDA levels. A single p.c. treatment with 10 µg PMA did not affect control levels of 25 pmol/µg DNA for up to 15 hrs after treatment. MDA content started to increase at 18 hrs, reached a maximum of 45 pmol/µg DNA at 27 hrs, and returned to control levels at 33 hrs. This study is being conducted in collaboration with Dr. Gisela Witz, Department of Environmental and Community Medicine, Rutgers Medical School.

The polypeptide, mellitin, exhibits many biological and biochemical effects similar to PMA. It was confirmed, for example, that mellitin, like PMA, is a powerful hemolytic agent for mouse RBC. In addition, gangliosides were found to inhibit the hemolysis by mellitin as well as by PMA.

The ability of mellitin to act as a promoter and as a promotion modifier was tested. Subcutaneous injections, but not percutaneous treatment, were inflammatory for the mouse skin, and mellitin was consequently given sc. Mice were initiated with DMBA and promoted with triweekly sc. injections of 50 µg mellitin. An additional experiment included PMA treatments. Mellitin produced no tumors after 210 days and did not markedly affect promotion by PMA.

Sponsor: National Cancer Institute, Grant No. CA 18536

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Publications:

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19. Early Biochemical Mechanisms of Tumor Promoters

Among the many *in vivo* and *in vitro* effects of phorbol myristate acetate (PMA) are the stimulation of cell growth and proliferation, and a concomitant increase in the rate of macromolecular synthesis. It is interesting to note that RNA synthesis, which occurs in the nucleus, is stimulated by PMA, although PMA has been postulated to be a membrane-active agent. At present, no clear mechanism has been found to explain the transduction of the PMA-induced signal from the membrane to the nucleus.

In an effort to understand the mechanism of PMA action, experiments were designed to clarify the time course of PMA action on RNA synthesis. Cells at varying stages of growth were treated with 0.5 μg/ml PMA for 24, 48, or 72 hours and then for one hour with [^{3}H]-uridine. Following incubation with the uridine, the rate of RNA synthesis was determined by measuring the amount of radioactivity in the RNA and in the acid-soluble fraction prepared from the cells (the nucleotide precursor pool). By normalizing the incorporation of label into RNA with respect to soluble activity, only specific RNA synthesis was measured, and other factors such as uridine transport or metabolism were excluded.

No effect by PMA was observed when RNA synthesis was measured in growing cells, regardless of the length of treatment. However, when cells were treated 24, 48, or 72 hours before reaching confluence, and RNA synthesis was measured in the confluent cells, a 60-100% increase in synthesis was found. Cells treated while confluent were not responsive unless medium containing serum was present. In one experiment in which growing cells were deprived of serum, RNA synthesis measured at confluence was not affected by PMA. It appears, therefore, that PMA requires the presence of undefined factors in serum to exert a stimulating effect on RNA synthesis. The same phenomenon has been reported for cell growth and DNA synthesis.

The PMA effect was dose dependent from 0.025 μg/ml to 0.5 μg/ml. A kinetic analysis of uridine uptake showed that neither total cellular uridine incorporation (which reached equilibrium at ~15 min) nor the specific activity of the soluble nucleotide pool were affected by PMA treatment.

When growing cells were incubated with PMA for 4 hours and then washed with fresh medium and allowed to multiply to confluence, brief exposure to PMA was as effective as 24 to 72-hour exposures in stimulating RNA synthesis.

The results suggest that the action of PMA as a stimulator of RNA synthesis (and other growth-related processes) involves a synergistic interaction with growth stimulating factors in serum. It seems unlikely, therefore, that PMA acts directly on the nucleus. The complexity of this repsonse is further indicated by the importance of the growth stage of the target cells.

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Garte, S.J. and S. Belman. Stimulation of RNA synthesis *in vitro* as a biochemical assay for the mechanism of action of phorbol myristate acetate (PMA). *Proc. Amer. Assoc. Cancer Res.* In Press.

20. Influence of Fiber Length on Fibrogenicity and Carcinogenicity of Inhaled Asbestos

The ability of inhaled asbestos to produce asbestosis, lung cancer, and mesothelioma in both human beings and animals is well established, and asbestos exposures in the occupational and general community environment are recognized as significant hazards. However, it has not been possible to establish credible dose-response relationships for asbestos exposures, primarily because of our inability to define which constituents of the aerosols initiate the pathological responses. It is generally acknowledged that the responses are associated with the fibers rather than the non-fibrous silicate mineral of the same chemical composition which is always present in any asbestos aerosol. It is also clear that fiber size is an important determinant of the toxicity of mineral fibers.

Intratracheal instillation and pleural implantation studies with suspensions of long and short fibers have demonstrated that fibers less than 10 to 20 μm long produce little if any fibrosis or mesothelioma, while longer fibers with similar diameter distributions and mass loadings produce high incidences of these diseases. It is important that similar studies be made by inhalation exposure, since inhalation is the major route of occupational exposure and an important route for general community exposure as well. Unfortunately, it has previously not been possible to generate suitable fibrous aerosols for such studies. A definitive study would require large volumes of fibrous aerosols with defined fiber lengths, aerodynamic diameters, and concentrations.

In this study, a combination of separation techniques is being developed that can be employed to generate fibrous aerosols with appropriate aerodynamic diameter and length distributions, and inhalation exposure protocols are being designed to use these classified fiber aerosols. Following the exposure of rats to the aerosols, the respiratory mechanical functions of the animals will be measured, and histological evaluations of the tissues of the animals will be made.

Two aerodynamically classified crocidolite asbestos aerosols will be produced whose aerodynamic diameters (D_a), fiber lengths (L_f) and concentrations (C) will be:

short fiber aerosol: D_a = 1.5 - 2.5 μm; $L_f \leq 5$ μm; C = 50 mg/m^3

long fiber aerosol: D_a = 1.5 - 2.5 μm; $L_f \geq 15$ μm; C = 250 fibers/cc (For 2 μm aerodynamic diameter, 25 μm long = 50 mg/m^3).

The basic concept is to have a continuous dynamic flow system which will utilize the aerodynamic properties of asbestos fibers to separate them into series of fractions according to their aerodynamic sizes and lengths. All of the separations will be performed while the fibers are airborne, and the aerosols produced will be fed directly into animal inhalation chambers.

To provide a continuous supply of the fibrous feed material, a leak-tight reservoir drum will be filled with enough fibrous raw material to permit continuous operation for at least 10 hours at the maximum feed rate contemplated. A mechanical conveyor will draw material from this reservoir and deliver it to the aerosol generator, and a fluidized bed generator will be used for generating a mixed aerosol of fibers and other particles with a wide range of sizes, which will flow continuously out of the top of the column.

A virtual impactor will project particles having aerodynamic sizes greater than the design cut-size (1.5 μm) into a small volume fraction of the original stream which flows down a central outlet tube. The bulk of the entering flow, containing most of the particles with aerodynamic sizes below the cut-size, will be drawn into the outer annulus and, from there, to a collection filter.

A cyclone will be used to remove particles and fibers having aerodynamic sizes greater than 2.5 μm. Since the virtual impactor removed the undersized particles, the airborne particles leaving the outlet of the cyclone will have a narrow range of aerodynamic sizes, with a mass median diameter of ~0.7 μm (AMAD ≈2 μm).

The final and key element in the system, the classification of the airborne fibers according to length, will be carried out by a device which separates the particles according to their electric mobilities. The separation is based upon the different mobilities of fibrous and more compact particles which pass through the charger.

A modified GCA fibrous aerosol monitor will be used for the periodic measurements of the fiber count concentration in specific fiber length increments. This instrument uses electromagnetic fields to rotate fibrous particles passing through the sensing zone of a laser photometer. The intensity of the characteristic scattered light pattern caused by a fiber within the sensing zone is a function of its length. Therefore, output from the instrument can be sorted and stored as fiber length distributions. The basic commercial instrument is normally set to count all fibers longer than 5 μm. With the addition of a pulse height analyzer, the fiber length distributions can be recorded as well. Sampling probes from the instrument will be located on each level of the exposure chamber.

The relatively rapid response of the instrument will permit the adjustment of the aerosol feed rate during the day and, therefore, the uniformity of the exposure. During the non-exposure hours, the instrument will monitor the fiber concentration in the air of the housing chamber, to characterize the concentration and length distribution of the fibers resuspended from the fur of the animals exposed during the day.

The fibrous aerosol monitor does not respond to fibers shorter than 2 μm and, in any case, the count of 2 to 5 μm long particles in the short fiber aerosol will not be indicative of either its total fiber

count or mass concentration. It will, therefore, be used only occasionally to characterize the number concentration of 2-5 μm long fibers. Since the short fiber chamber concentration will be maintained at a constant mass concentration, the routine monitoring of the chamber concentration will be done using a GCA Real-Time Aerosol Monitor.

The mass concentration of asbestos dust in each chamber will also be determined gravimetrically by collecting samples on filters. Additional samples will be analyzed by phase contrast microscopy to provide fiber concentrations in the form comparable to that used to evaluate occupational exposures, and to check on the performance of the GCA Fibrous Aerosol Monitor.

As soon as all of the preliminary work is completed, an exploratory 90 day inhalation exposure study will be started. Two groups of 54 male, 9-week old, SPF Fischer rats and a similar number of controls will be exposed to each of the two aerosols or to a sham exposure environment. Measurements will be made of dust retention and tissue response following serial sacrifices of 5 rats at 1, 5, 10, 30, and 90 days during the exposures, and at 90-day intervals following the end of the exposures. Tissue response measurements will include determination of total collagen content in proportion to total protein content, ^{3}H-proline incorporation into lung protein, and the activity of pulmonary prolylhydroxylase. The survivors will be evaluated periodically for respiratory mechanical function, and alterations of function will be related to the progression of fibrotic changes, measured biochemically.

Finally, a lifetime inhalation exposure study will be carried out in which eighty-one male, 9-week old, SPF Fischer rats will be exposed initially to each of the two aerosols. After one year, the numbers of exposed animals will be reduced to 54 in each group, and the remainder will be removed from exposure and studied separately, with focus on their changes in pulmonary function. In addition, 19 rats will be housed in the same housing chamber as "family members" of each group of exposed rats. They will receive a low level of exposure from dust resuspended from the fur of the exposed animals. None of these animals will be sacrificed, but all will be examined for fibrosis and tumors following their natural deaths.

Sponsor: National Cancer Institute, Grant No. CA 26724

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21. Purification of Mouse Epidermal Nuclei

Studies are being conducted on the characterization of nonhistone nuclear proteins (NHNP) in normal and cancerous mouse epidermal nuclei. It was, thus, necessary to isolate mouse epidermal nuclei free of adherent cytoplasm and other contaminants. Previously reported attempts by others to isolate mouse epidermal nuclei have yielded preparations contaminated by keratin and other debris. The isolation and purification of mouse epidermal nuclei was accomplished by homogenizing epidermis in 3% citric acid. A large portion of nonnuclear material was then separated from the nuclei by filtering through a series of microporous nylon mesh filters. Final purification of nuclei from cellular debris, and from nuclei retaining traces of adherent cytoplasm, was achieved by banding the nuclei in a Metrizamide density gradient. The procedure resulted in the recovery of approximately 2.8×10^6 nuclei per mouse, which was 39% of the nuclei originally present in the epidermal homogenate. The nuclei banded at a density of 1.30 gm/ml in Metrizamide. The protein, DNA, and RNA contents of the nuclei were determined by be 47.2, 7.4, and 7.0 pg per nucleus, respectively.

Sponsor: Monsanto Fund Fellowship in Toxicology

Principal Investigator: A. Segal, Ph.D.

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22. Temporal Aspects of Tumorigenic Response to Individual and Mixed Carcinogen Exposures in Relation to Risk Assessment

The research described here is designed to obtain a better understanding of the temporal kinetics of tumor induction, when one or more carcinogens are present simultaneously or sequentially for prolonged periods of time. Studies done to date have shown that carcinogenesis in mouse skin by polycyclic aromatic hydrocarbon carcinogens is consistent with the induction of dependent and autonomous cell transformation by the carcinogen, followed by conversion of the autonomous tumor cells into malignant cells at a rate determined by the level of carcinogen or promoter exposure. Dependent cell transformations may remain latent in the skin if not stimulated by a promoting agent. Dependent neoplasia appears to follow one-hit kinetics, while malignancy is a multi-hit endpoint. Dose-related and time-related aspects of tumor induction are separable in the initiation-promotion system of mouse skin which, along with rat skin, is being used as a model for testing hypotheses. Results, to date, provide the basis for a new interpretation of the linear non-threshold extrapolation model. The broad aim of the study is to provide a rationale for estimating risks associated with prolonged exposures to carcinogens found in the environment, and to predict how different

tissues and species respond to carcinogens, promoters, and cocarcinogens.

Mouse skin has been extensively utilized as a test system for carcinogenicity, and this laboratory is attempting to refine our understanding of it so that it may be more readily used for quantitative risk assessment of various types of compounds, including initiators, promoters, and cocarcinogens. Recent results suggest that several types of initiating events may occur as a result of exposure to polycyclic aromatic hydrocarbon carcinogens. Some of these events lead to progressing tumors that will eventually become carcinomas. Both progressing or non-progressing papillomas were produced in proportion to the carcinogen dose applied. The carcinomas that arose from progressing papillomas were also produced in proportion to dose.

Promotion greatly increased the rate of formation of carcinomas in comparison to exposure to a carcinogen alone. The higher yield of cancers may be the result of clonal expansion of papilloma cells having a potential for progression. Progression to cancer occurs in the absence of a promoter, but a much higher dose of carcinogen is needed and the yield is a curvilinear rather than linear function of dose. In the absence of a promoter, progression to cancer requires 4 to 5 times more carcinogen than when a promoter is present. Furthermore, the presence of a promoter changes a curvilinear carcinogen dose-response curve into one that is linear.

The outcome of a given exposure and the method to extrapolate to low doses may depend critically on the relative mixture of initiating and promoting activity. These classes of compounds are clearly not distinctive, e.g., benzo(a)pyrene has demonstrable promoting activity despite its strength as an initiator. Current experiments have three main purposes: 1) to clarify the manner in which initiators and promoters interact to produce a given temporal pattern of tumor induction, 2) to characterize more clearly the "dose" of these agents and how different tissues and organisms react when compared on the basis of intracellular "dose", and 3) to define more exactly the nature of cocarcinogenesis. Such information will be extremely useful in comparing different species and organs and in making extrapolations from high doses to low doses, where information is badly needed for making regulatory decisions.

Information on the autonomy of induced neoplastic lesions was derived from experiments where the carcinogenic or promoting chemicals were applied for a certain time and then stopped. To study the carcinogenic effect of specific periods of exposure, three types of experiments are being conducted: 1) a single dose of the initiating chemical, benzo(a)pyrene, 7,12-dimethylbenz(a)anthracene or beta-propiolactone is applied, followed by promotion for a specific period of time, 2) various amounts of initiating chemicals are applied weekly for various periods up to the appearance of carcinomas, and 3) various amounts of the initiating chemicals are applied as in 2 with the addition of weekly doses of a promoter. It can be said at this time that the dose-response relation-

ship for mouse skin initiation by B(a)P is consistent with a linear non-threshold model, as is the dose-response relationship for B(a)P binding to DNA in mouse skin. The dose-response relationship for chronic lifetime exposure to B(a)P can be expressed as a power function of dose with the power between 2 and 3. However, when B(a)P is combined with phorbol ester in a chronic exposure mode, the dose-response relationship is changed to a linear non-threshold character.

It was found that B(a)P applied topically to mouse skin interacts with epidermal DNA to produce two major peaks (peaks 1 and 3) when analyzed by Sephadex LH-20 and high-pressure liquid chromatography. Preliminary evidence on repair indicates peak 1 to be nonrepairable, and peak 3 to be only slowly repairable (halftime 85 hours).

Experiments are in progress to determine if exposure to a carcinogen during various stages of fetal development results in the same kinds of "initiated" cells as does exposure of adult cells. The results so far indicate that fetal skin cells are much more likely than adult cells to be initiated by 7,12-dimethylbenz(a)anthracene. Even at 9 days of gestation, the dose-response curve for initiation was linear at the lower maternal doses.

Promoters may be significant carcinogenic hazards because they increase risk by shortening the time required for the cancers to develop. Such an increase in risk is no less important, and may not be distinguishable from an increase produced by the induction of new lesions. At the present time, there is very little information on the form of the dose-response relationship for promoters. Attempts are being made to define the dose-response relationship for TPA more carefully and to study the dose-response relationship for other promoters, especially anthraline.

The inhibitory action of a retinoid (retinyl acetate) on 7,12-dimethylbenz(a)anthracene-induced rat mammary cancer has been studied. It was found that relatively short periods of exposure to the retinoid can be inhibitory, and that the temporal displacements are consistent with having, in effect, reduced the carcinogen dose.

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23. Radiation Carcinogenesis in Rat Skin

Rat skin has proved to be a sensitive and reproducible model for studying the mechanism of radiation carcinogenesis, and for formulating ideas that could lead to improved estimates of risk in radiation protection calculations. Studies of the shape of the dose-response curve, the effect of spatial distribution of dose, the effect of the time pattern, and the importance of the density of energy deposition on the induction of skin tumors has led to a better understanding of radiation carcinogenesis. Whether tumor incidence is proportional to the amount of tissue irradiated was studied by irradiating rat skin in grid and sieve patterns of various pore sizes with electrons, protons, and low energy x-rays. A consistent finding was that localized dose patterns were less oncogenic than uniform patterns for low linear energy transfer (LET) radiation, electrons, and x-rays, but not for high LET protons. It was shown that radiation must penetrate to a depth of about 0.3 mm into the skin in order to induce tumors, suggesting that follicular stem cells may be the primary targets for oncogenesis. Skin is capable of repairing much of the radiation induced damage that leads to the formation of

tumors. Recovery from the oncogenic effect of electron radiation was demonstrated to have a halftime of 2 to 3 hours.

a. The Induction and Repair of DNA Strand Breaks

The dependence of DNA strand breaks (alkali-labile sites) on radiation dose and the rate of repair of such breaks was determined by means of an alkaline unwinding technique. Epidermal DNA was labeled prior to irradiation by giving intraperitoneal injections of ^{3}H-thymidine. After irradiation, the epidermal cells were removed and incubated in alkaline solution (pH = 11.4). After the incubation in alkali, S_1 nuclease was added to digest the single-stranded portion of the DNA. The amount of undigested, double-stranded DNA was determined by using a liquid scintillation counter to count the ^{3}H in the precipitated DNA. Plots of percent double stranded DNA versus time in alkali, referred to as unwinding curves, were utilized to estimate the number of strand breaks (alkali-labile sites) per unit DNA. The strand breaks were found to increase in proportion to radiation dose within the 300 to 2400 rad dose range employed in the experiment. When the skin was given 1200 rads, the strand breaks were found to be removed with a halftime of about 17 min. This halftime implies a rate of removal much too rapid for single strand breaks to be the major repairable component of carcinogenic injury, since the latter was found to be removed with a halftime between 120 and 180 min.

b. A Chromosomal Break Model of Radiation Carcinogenesis

A theory has been elaborated that attempts to explain radiation carcinogenesis in terms of interactions between broken chromosomes. The dose-response relationships for low LET electrons and high LET argon ions, as well as the effect of fractionation and dose rate, are consistent with the results of the model. The idea is that cancer induced by radiation is related to the formation of chromosomal rearrangements derived from the fusion of the broken ends of two separate DNA double strand breaks. The same mechanism is assumed to apply whether the radiation is densely (high LET) or sparsely ionizing (low LET). The explanation of the LET dependence is based upon the geometrical consequence of the breaks being produced either in the same track (leading to a linear term) or in different tracks (leading to a dose-squared term). At high LET, the tracks are so widely spaced that the interaction of breaks from different tracks is unlikely. Conversely, at low LET, the individual ionizations are so widely spaced along a given track and the number of tracks is so much greater for a given dose, that the interaction of breaks from different tracks is greatly increased in comparison to intratrack interactions. Furthermore, the breaks are assumed to be repairable, so that an interaction may be averted if the first of a potential pair is gone at the time the second break occurs.

It seems likely that double strand break fusions of the type envisioned here are extremely common in irradiated cells. As many as 20% of

all cells irradiated with 500 rad may exhibit chromosomal aberrations of the type involving such fusions. A high proportion of such aberrations are probably lethal, while others may be completely benign, and cells containing them may remain viable for many cell generations. Among the latter, the proportion undergoing neoplastic transformation could be extremely small.

c. Tumor Yield for Multiple Small Doses of Electrons

Previous studies have shown that when polycyclic aromatic hydrocarbons are applied to the backs of rats weekly, the tumor yield increases steeply, as the 5th or 6th power of time. In contrast, the time exponent for tumors induced by irradiation is only one or two, when the radiation is applied as a single dose. It is important to determine whether radiation given weekly for indefinite periods of time increases the time exponent, so that it becomes more similar to that seen in chemical carcinogenesis.

At the present time, rats have been given total doses of 1300, 2600, 5200, or 10,400 rads in 52 weekly irradiations. There has been no ulcerative damage to the dorsal skin that would indicate a cytotoxic effect. No tumors have yet appeared in the two lower dose groups, one tumor appeared at 40 weeks in the next to highest exposure group, and 5 tumors have appeared in the highest dose group, starting at 36 weeks. These results indicate that when electron radiation is protracted in this way, the rat skin is able to tolerate much higher doses than if the radiation were given in a single dose. It cannot be determined at this time, however, whether the protraction has affected the exponent of the time curve.

d. BUdR Sensitization of Rat Skin to Electron Tumorigenesis

This laboratory has shown that sufficient quantities of 5-bromo-2'-deoxyuridine can be incorporated into the DNA of rat epidermal cells to sensitize them to electron radiation, as measured by increased DNA strand breakage. The purpose of these experiments was to use these findings to determine whether treatment with BUdR will sensitize the rat epidermis to the carcinogenic effect of electron radiation.

Irradiated groups of rats, given either 80 or 160 mg of BUdR, showed an increased oncogenic response to 900 rads of electrons, compared to irradiation alone. At 1800 and 2100 rads, the BUdR treatment inhibited the tumor response, possibly because both 1800 and 2100 rads are near or beyond the peak tumor yield. The combination of BUdR with either 1800 or 2100 rads might cause enough cell lethality to decrease the number of cells at risk for radiation carcinogenesis.

e. The Dose-Response for Tumor Induction at Low Doses

The linear plus quadratic dose-response model ($Y = AD + BD^2$) has been used to extrapolate risk assessment to lower doses of ionizing

radiation. Presently, there are few data for evaluating A and B or the effects of dose rate and recovery on the basic equation. An experiment is in progress to determine the coefficient of the linear term (A) in the dose response equation, on the basis that if sufficient time is permitted between the doses of radiation, multiple doses should produce the summation of their individual effects. This experiment is based on the finding of previous experiments that the recovery halftime of radiation oncogenicity is about 2.5 hours. Therefore, 24 hours between doses should be sufficient for nearly complete recovery to occur. The results, so far, indicate that the exponent of the dose-response function is at least 2. The most probable value is 2.6, and no linear term was found even for a dose of 150 rads.

f. Epidermal Cell Survival in Rats and Mice Compared

Mouse skin is a traditional animal system for examining chemical carcinogenesis, but it has been found to be somewhat refractory to the oncogenic effect of ionizing radiation. The purpose of these experiments was to measure the acute lethality to the epidermal cells of several strains of mice and rats, of both ionizing and non-ionizing radiations, and chemical carcinogens, such as 7,12-dimethylbenz(a)anthracene. The species and strain comparison was made with C57Bl and Balb-C mice and CD-1 rats.

The animals were given intraperitoneal injections of 2 μCi/g body weight ^{3}H-thymidine (40-60 ci/mmole; Amersham), to label DNA. Two hours after injection, the animals were exposed to various doses (0, 400, 800, 1200, or 1600 rads) of 0.8 MeV electrons at a dose rate of 600 rads/min. Twenty-four hours after irradiation, a skin biopsy was taken from the irradiated area, and an autoradiograph was made of the epidermis. Any cell that incorporated ^{3}H-thymidine and then underwent mitosis appeared as a pair of adjacent labelled cells. Defining the lack of labeled pairs as a lethal event, survival curves of the epidermal cells were constructed. The high dose slopes were essentially the same for all strains tested, but there were considerable differences at low doses. The results were consistent with the existence of a subpopulation of cells in the mice with special sensitivity to the lethal effects of the radiation. The greater sensitivity of mouse skin cells to the lethal effect of radiation could explain the lower sensitivity of mice to the carcinogenic effect of the radiation, in terms of a reduction in the number of cells at risk.

g. DNA Strand Break Repair as a Function of Age

It has previously been shown by this laboratory that the ability of rat epidermal cells to repair electron-induced DNA strand breaks decreases as a function of the age of the animal. The halftime of repair increased from 21 min in 28 day old rats, to 69 min in 200 day old rats, to 109 min in 400 day old rats. This result is being utilized in studies to determine whether this age-related impairment could be influenced

totally or partly by exposure to deleterious environmental agents, such as ultraviolet light.

Rats at 28 days of age are being exposed to single (0, 8 x 10^5 and 13.6 x 10^5 ergs/mm^2) and weekly (0.4 x 10^5 ergs/mm^2) doses of solar spectrum (275-375 nm) ultraviolet light. At various ages, the dorsal skin will be assayed for the repair of DNA strand breaks induced by a standard dose of 1200 rads of 0.8 MeV electrons. Early effects of UV exposures will be examined by measuring DNA repair 2-30 days after UV exposure, while late effects will be examined 100-400 days after UV exposure.

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C. RADIATION DOSIMETRY AND CARCINOGENESIS

1. Determining the Magnitude and Position of a Gamma-Emitting Source, Using the Principle of Active Collimation

Investigations are proceeding on an advanced type of gamma detection system which uses a series of interleaved NaI(Tl) and CsI(Tl) wafers, each 7.6 mm thick, to provide magnitude and positional data.

The NaI(Tl) elements, located at the detector center and sides, are recessed with respect to the CsI sections. This prevents their illumination except by photons whose incidence angle is almost normal to their surfaces. A common PM tube is suitably masked and positioned with respect to the wafer assembly, so that controlled non-uniformity of illumination, in combination with scintillator behavior and amplified characteristics, produce separate spectra from each wafer without overlap.

Response of the prototype detector was obtained by mounting it in the moveable arm of a Nuclear Chicago Pho-Dot scanner, which was converted to operate as an incremental positioning device. The dedicated Nova 2 computer in a Tracor Northern 1700 pulse height analyzer was modified to provide an actuation trigger, under control of a stored program, to a position control unit which produced successive 5 mm incremental movements of the detector arm. The position control unit, following each repositioning of the detector, indicated the new location of the detector's center by a mark electronically struck on a coordinate grid. This allowed precise determination of the detector's position with respect to the radioactive source at each point in its travel. A 300-second count was taken at each station, after which the stored computer program provided on-line typeout of location number, individual wafer counts corrected for background and spectral interferences such as I and Cs XRF and comptom scatter, and the multiple products, with propagation of error, resulting from left and right-side wafer counts. The process was then automatically repeated under computer control for each detector position. Since the field to be scanned was to cover, at a minimum, the domain of a 3 x 3 detector array, or 3 detector dimensions (38.1 mm square) an area of approximately 144 cm^2 was surveyed in 5 mm segments along the abscissa and ordinate axis, producing a block of data comprising over 700 detector locations.

The data thus obtained were then utilized to derive quadratic curve-fit functions that were incorporated into a computer program to synthesize the response of a 3 x 3 array of variously aligned detectors. A parquet wafer pattern results, with each detector being rotated 90° with respect to the wafer alignment of its neighbor. This program, written in BASIC, utilizes all available locations of a 24,000 word memory and allows the detector array to scan, in any size increments, one or more sources in any desired configuration and coordinate position. Each source is viewed, in turn, by each detector. The absolute and relative positions of a detector with respect to each source point are

printed, along with the individual and cumulative wafer responses, and multiple products, as a function of X and Y displacement.

The study of the response of the detector array to different source configurations, including line segments, arcs, and solid surfaces is currently under way. Initial results confirm expectations that the detector is extraordinarily responsive to small position changes, e.g., when a line segment represented by 3 point sources 25 mm apart is scanned, the product output of the approaching detector increases from 0 to 3.8 x 10^8, within a distance of only 2 mm. This sudden and pronounced change in output is a result of the modulating effect occurring when a recessed NaI side wafer, by only miniscule movement, comes into a position where it is just barely illuminated by photons emanating from the edge of a source. It is anticipated that the subsequent analysis of data will reveal an exact, intrinsic relationship between source edge and detector position, where a sudden change in response slope is discernible. By utilizing slope change rather than absolute count data, it will hopefully be possible to produce a detector response that is relatively independent of background and count rate.

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2. *In Vivo* Measurements of Actinide Contamination in the Lung: Correction for Skeletal Interference

Sensitive methods for the routine surveillance of occupational personnel are essential for identifying the possibility of internal contamination and, if exposure has occurred, for designing effective protocols for decorporation therapy. In addition, it is necessary to be

able to identify and determine the magnitude of internal contamination for both occupational and non-occupational exposures.

In vivo measurement of internally-deposited radioactive material is becoming increasingly valuable as an adjunct to the more routine bioassay programs for determining the degree to which an internal exposure has occurred. It is also useful for the determination of the deposition parameters needed to accurately calculate the radiological dose to a particular organ or tissue.

Since the predominant occupational exposure to transuranic nuclides often involves the inhalation of long-lived, non-transportable materials, internal contamination is usually quantitated with detectors placed above the lung area over the anterior thorax. Many of the internally-deposited alpha-emitting nuclides, however, are bone seekers (i.e., they concentrate primarily within or on the surfaces of the skeleton). Therefore, it is necessary to account for the contribution of material which has been transported from the lung to the thoracic skeleton. For this reason, a method has been developed where lung-deposited radioactivity can be more accurately estimated by subtracting the thoracic skeletal contribution from a chest count. Otherwise, the potential error occurring in the estimation of a lung burden can be considerable when there is a significant radionuclide deposition in the throacic skeleton.

The technique for estimating the magnitude of this contribution is performed by surrounding the head with three NaI(Tl)-CsI(Tl) dual crystal scintillation detectors to determine the activity deposited in the skull. A standard anterior thorax measurement is then made using two NaI(Tl)-CsI(Tl) detectors positioned over the chest. To obtain all the information needed to estimate the final lung burden, one must know the calibration values for the head, the lung, and the thoracic skeleton. These values can be determined from the measurement of three phantom structures which represent these specified areas of the body.

Thus far, construction of lung and skull phantoms have been completed for the nuclides ^{241}Am, ^{239}Pu, ^{238}Pu, and ^{210}Pb. In addition, a complete articulated americium thoracic phantom completed last year has been successfully utilized to estimate the contribution of ^{241}Am in the thoracic skeleton to lung counts of three exposed individuals. Construction of an articulated plutonium thoracic phantom is currently underway. Based on the phantom measurements, minimum detectable true activity values for plutonium, americium, and ^{210}Pb have been calculated for the skull bone deposition site. At the 95% confidence level for 100-minute subject counts, these values were determined to be 2.37 nCi for ^{239}Pu, 0.96 nCi for ^{238}Pu, 180 pCi for ^{210}Pb, and 21.9 pCi for ^{241}Am.

Further research has been performed to optimize counting geometries in an effort to define a site which will give maximum efficiency and minimum count-rate variability, while allowing for long-term counting times with minimum inconvenience to the measured subject. Measurements

have been obtained at the thorax sides with arms elevated above the head or with the detectors placed over the posterior thorax.

To upgrade whole body data processing capabilities, an on-line interactive computing system has been added to the existing facility. The major addition to this system provides a Telex link to the CDC 6600 computer at the Courant Institute of Mathematical Sciences (CIMS). With this addition, it is now possible to make use of the extensive existing software at CIMS.

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3. Mathematical Modeling of the Uptake, Retention and Distribution of Uranium in the Primate

The objective of this research has been to develop a model that can be used to calculate the accumulation of uranium in the different organs of the human body for various kinds of exposure.

The proposed model divides the human body into compartments -- plasma, red cell, "short-term" bone, "long-term" bone, kidney, and urine. The rate of transfer between compartments is assumed to be governed by first order kinetics, and transfer from plasma to the other compartments and red cells to plasma is taken into account.

The division of blood into plasma and red cell compartments is an improvement over existing models, and it has proved to be important for calculations of uranium transport during the first few days after exposure. In addition, it has been noted that uranium in bone may be represented as having two different half-lives depending on the site of deposition, a "short-term bone component" and a "long-term bone component".

An exact analytical solution to the model was proposed that can be solved for any kind of time-dependent exposure to uranium. This methodology is unique to this model, and it represents a significant change in analytical solutions that have been proposed in the literature.

Specific analytical solutions for the more common cases of uranium exposure were derived which include:

1. single injection dose to the blood,
2. exposure to background levels of natural uranium by ingestion,
3. exposure through inhalation during working hours for uranium workers,

4. single inhalation dose (most probable case for accidents),

5. constant inhalation exposure during a finite interval of time, and

6. single ingestion dose.

In this study, five baboons were injected intravenously with uranium nitrate, and the partition of uranium between plasma and red cells was studied. The half-life in "short-term" bone was derived. Examination of the distribution of uranium in soft tissues four days after injection showed that the kidney was the major organ for uranium deposition. The concentration in skeleton was calculated using a gastrointestinal absorption factor of 23% and a daily excretion value of 24 μg U/day.

This model is suitable for deriving dose limits and for determining body burdens from standard bioassay information.

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Skrable, K.W., G.E. Chabot, C.S. French, M.E. Wrenn, J. Lipsztein, T. Lo Sasso and P.W. Durbin. Blood-organ transfer kinetics. *Health Phys.* *39*(2):193, 1980.

4. X-Ray Dose to the Skin of the Head and Neck in Patients Irradiated for Tinea Capitis

The x-ray dose to 30 locations on the skin of the head and neck was measured in a reconstruction of an Adamson-Kienbok tinea capitis treatment. This was done by utlizing the phantom used by this laboratory in previous tinea capitis dose reconstruction studies. The phantom is made from the skull of a seven year old child, and it is covered with a tissue equivalent wax (MIX D).

So far, in epidemiological studies, eighty-two basal cell carcinomas (BCC) have appeared in 40 of 1727 irradiated white children, and none in 500 irradiated black children. The dose response per unit area of irradiated skin over the face and scalp was used to estimate the risk of BCC per person per rad.

The results are limited because of the relatively young age (< 43 years) of the irradiated group at the present time. However, it appears that the risk of BCC per rad is decreased on the hair-covered scalp relative to that on the face. This incidates that environmental ultraviolet radiation may play a key role in the expression of BCC.

An increased risk of BCC has been reported in Czechoslovakian underground uranium mines. The BCC risk there is thought to be related to alpha emitting radon daughters deposited on the skin surface, and attempts are now being made to verify the alpha dose factors reported in that study.

If the data are substantiated, environmental levels of radon daughters could deliver an alpha dose of one-half rad per year to basal cells in the epithelium.

Sponsor: Institute of Environmental Medicine

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5. Measurement of X-Ray Exposure from Television and Word Processor Screens

A study was performed to test the feasibility of measuring the low-level x-ray emissions from television and word processor screens using thermoluminescent dosimeters (TLD). A few such measurements have been performed, and exposures of the order of 0-0.5 mR per hour have been reported in the literature.

This laboratory had previously developed a technique using Harshaw TLD-700 ribbon to measure a few milliRoentgens of environmental gamma ray exposures to within ± 5%, and this technique was applied to the measurement of the x-ray emissions from cathode-ray tubes. However, because of the low x-ray energy involved, extensive calibrations in the low energy x-ray domain were also performed. This was accomplished with the cooperation of the Department of Dermatology of the New York Univer-

sity Hospital. A calibrated Grenz ray dermatologic unit was used to determine the TLD response from effective x-ray-energies of 6-30 keV.

Dosimeters were prepared which could be taped to the face of the cathode ray tube, and each packet contained six TLD-700 dosimeters. In this way, duplicate measurements could be made with no added absorber and with one and two layers of 60 mg/cm^2 polycarbonate foil over the TLD ribbon. Using the absorption data, it was then possible to estimate the half value layer of the x-ray radiation and, thus, its effective energy. Control dosimeter packets were placed near the cathode-ray tubes to monitor natural environmental gamma ray background.

Preliminary measurements indicate that somewhat less than 0.5 mR per hour is actually present at the face of these monitors whether or not the cathode ray tube is on. Therefore, the probable source of this exposure is ^{40}K in the glass tube envelope itself and natural radionuclides in the phosphors used on the inside surface of the screen.

Sponsor: NIOSH Fellowship

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6. Long-Term Measurement of Environmental Levels of Radon for Estimating Population Exposures

A model to predict lung cancer incidence and lifetime lung cancer risk from environmental levels of radon daughters was developed. This model utilizes the underground uranium mining lung cancer experience at high levels of radon daughter exposure to estimate effects at environmental levels. The model can account for the observation that there is a higher lifetime lung cancer incidence when first exposure occurs at an older age, by correcting a uniform risk expression with an exponential decay factor ($t_{\frac{1}{2}}$ = 20 years) from the time of exposure. The model fits the U.S. and Czechoslovakian underground lung cancer experiences well. The predictions for environmental levels of radon daughters include the slight differences in alpha dose to the bronchial epithelium which occur between a mining atmosphere and environmental atmospheres. The model predicts that the lifetime lung cancer risk for an individual is 3.6 x 10^{-6} per pCi per m^3 of ^{222}Rn (daughter ratio 1/.9/.7/.7 and 7% unattached RaA).

Long-term population exposures to radon and its daughters are not well documented. Some short-term measurements have been reported in a few regions of the United States, but the relationship between these measurements and the average annual population exposure in a given

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VI. INDEX TO STUDIES BY PRINCIPAL AND CO-INVESTIGATOR