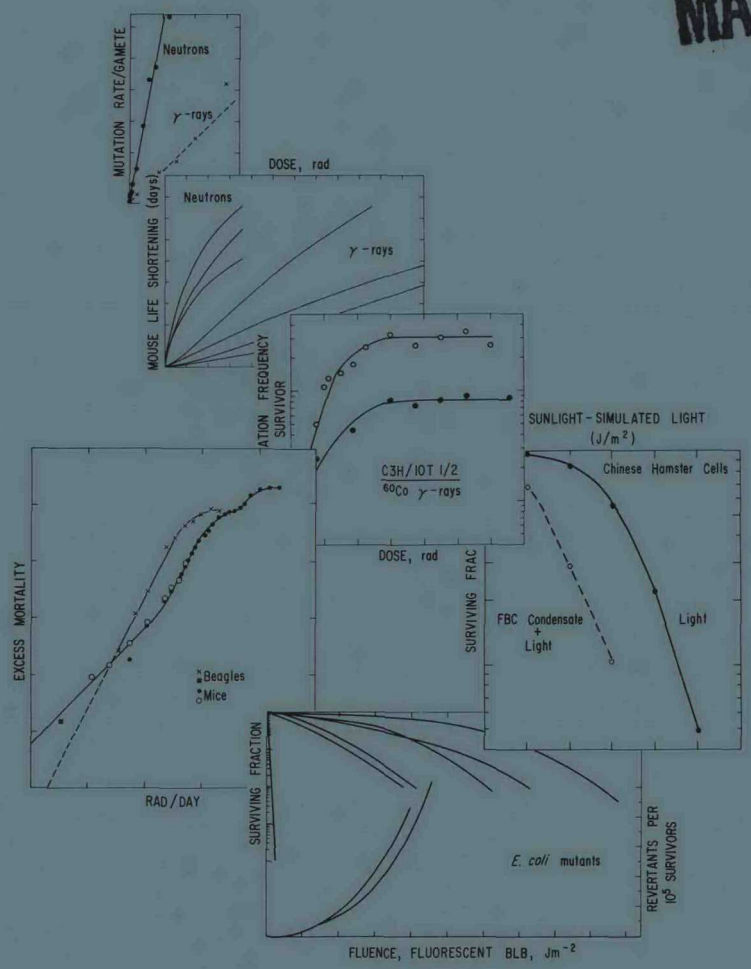
ANL-80-90

DIVISION OF BIOLOGICAL AND MEDICAL RESEARCH

Annual Report 1979



ARGONNE NATIONAL LABORATORY

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DIVISION OF BIOLOGICAL AND MEDICAL RESEARCH

ANNUAL REPORT 1979

Douglas Grahn, Director
Steven S. Danyluk, Associate Director
Thomas E. Fritz, Associate Director
Marcia W. Rosenthal, Editor

Preceding Report
ANL-79-90, December 1978

C O V E R

MONTAGE OF DOSE RESPONSES FOR DIVERSE END POINTS, ANIMALS, AND RADIATION SOURCES, REPRESENTING THE DIVISION'S RESEARCH ON THE EFFECTS OF IONIZING AND NON-IONIZING RADIATIONS.

ABSTRACT

The research during 1979 in the Division of Biological and Medical Research, Argonne National Laboratory, is summarized. Studies related to nuclear energy include responses of beagles to continuous low-level ^{60}Co gamma radiation, and development of indicators of leukemia ; comparison of lifetime effects in mice of low-level neutron and ^{60}Co gamma radiation; genetic effects of high LET radiations; and studies of the gastrointestinal absorption of the actinide elements. Studies of nonnuclear energy sources deal with characterization and toxicological evaluation of effluents of fluidized bed combustion and coal gasification; electrical storage systems; and electric fields associated with energy transmission. Assessment of human risk associated with nuclear as well as nonnuclear energy systems is approached through development of data bases and dose-response and demographic models. Basic research includes studies of fundamental structural and biophysical investigations; circadian rhythms; mutagenesis in bacteria and mammalian cells; cell killing, damage, and repair in mammalian cells; carcinogenesis and cocarcinogenesis; the use of liposomes as biological carriers; and studies of environmental influences on life-span, physiological performance,and circadian cycles. In the area of medical development, proteins in urine and tissues of normal and diseased humans are analyzed, and advanced analytical procedures for use of stable isotopes in clinical research and diagnosis are developed and applied. The final sections of the report cover support facilities, educational activities, the seminar program, staff talks, and staff publications.

TABLE OF CONTENTS

1. Division Director's Introduction

This report is concerned with the progress of research in the Division of Biological and Medical Research during the calendar year 1979. It departs from the usual procedure, an essentially de novo description of the principal activities during the year, in that it is based primarily upon the program summaries prepared early in 1980 for the Argonne Universities Association Review Committee. The program summaries include the principal activities over a period of approximately two years, in a few cases more, in order to provide better continuity and perspective for the current accomplishments.

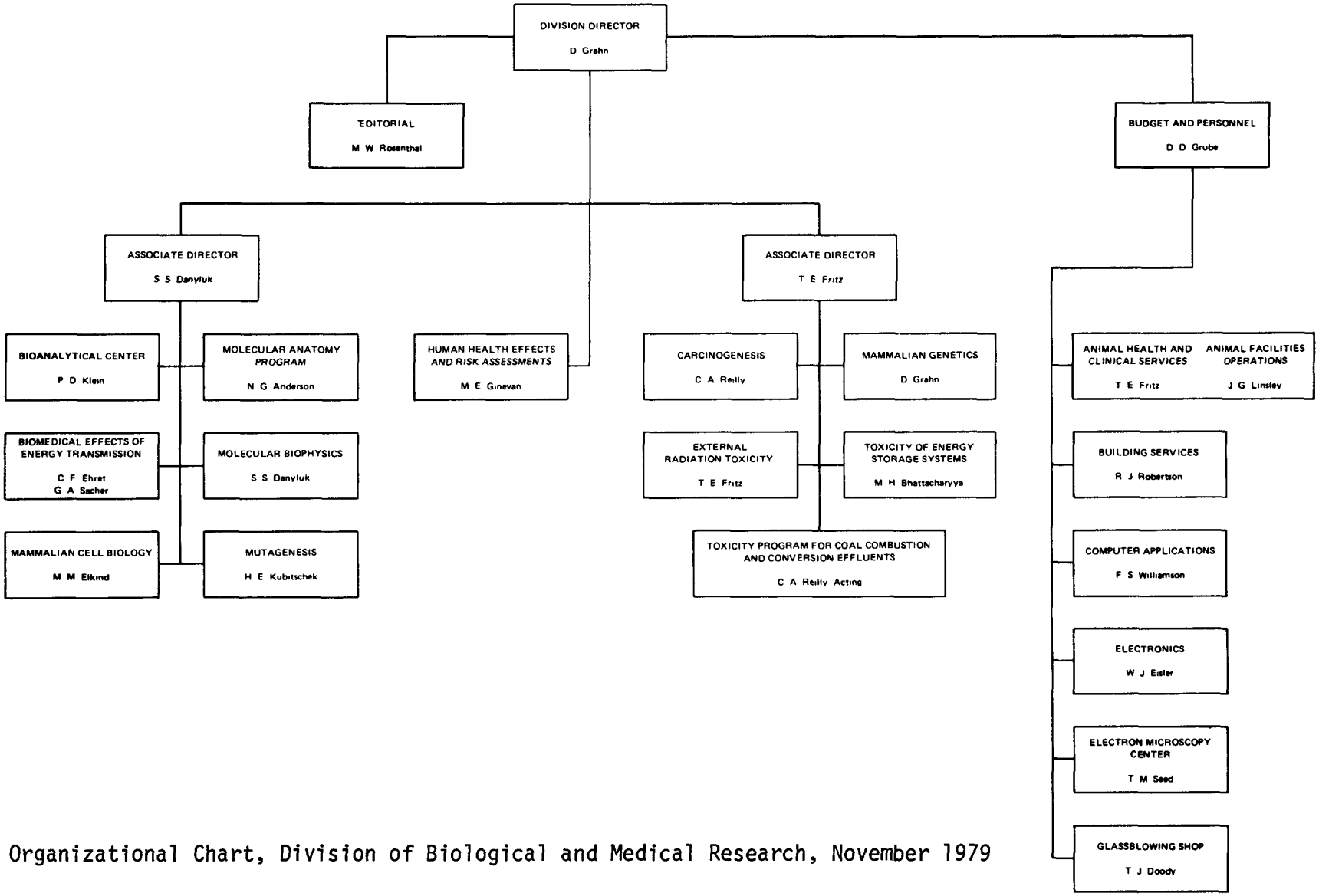
The 14 scientific chapters are based upon the scientific groups in the Division structure, as shown in the following organizational chart, with the addition of a section on Environmental Physiology and one on Liposomes as Biological Carriers. Collaborations among groups are frequent, as indicated by cross references in the text and by report authorships.

The last five sections of this report indicate the range of other activities in the Division. They present the major support facilities, the varied graduate and undergraduate level educational activities, the formal and informal Divisional seminars, staff seminars and presentations at scientific meetings, and a list of staff publications appearing during the calendar year 1979.

Two organizational changes were made in the Division during the year. First, Dr. Christopher A. Reilly, Jr. was named Acting Group Leader of the Toxicology Program for Coal Combustion and Conversion Effluents, on the retirement of Dr. William P. Norris. Second, the three former groups dealing with the toxicity of external radiations--Neutron and Gamma-Ray Toxicity Studies (the JANUS project), Radiation Toxicity in Dogs, and Cellular Indicators--were combined into a single group, the External Radiation Toxicity Group. This reorganization will bring about more cohesiveness in the Divisional programs involving the late effects of external radiation in mammals. Dr. Thomas E. Fritz was named group leader of the new group and also Associate Director of the Division. These changes free Dr. John F. Thomson, formerly Group Leader of the JANUS project and Associate Division Director, of his administrative duties, which he has performed so ably for the Division over the last 10 years, under several titles, and they enable him to devote full time to research.

An addition to this year's report is a listing above each report of the agency supporting the research during the calendar year 1979. Given are the U. S. Department of Energy (DOE) budget category, the DOE budget and reporting number, the "ANL number," and appropriate information regarding support from outside agencies.

Douglas Grahn, Director
Division of Biological
and Medical Research



Organizational Chart, Division of Biological and Medical Research, November 1979

2. External Radiation Toxicity

T. E. Fritz

During 1979, three Divisional groups--Neutron and Gamma-Ray Toxicity Studies, Radiation Toxicity in Dogs, and Cellular Indicators--were combined into a single group, the External Radiation Toxicity Group, in order to bring more effective interaction to the research involving the effects of external radiation in mammals. The program characterizes the late efects of low doses of ionizing radiation given to experimental animals in order to obtain information that will (1) be of predictive value in assessing hazards in man, and (2) enhance the understanding of radiation damage. Fractionated, protracted, and continuous radiation are given to two species of rodents and to beagles. Life shortening, causes of death, numbers and types of neoplasms and other induced lesions, as well as changes in cell ultrastructure and the kinetics of cell maturation and differentiation are used to measure the radiation damage.

The program consists of the three research areas mentioned above, and described in the following three sections.

REGULAR STAFF

Katherine A. Allen (Scientific Associate)
*Emily J. B. Christian (Scientific Associate)
*Rosemarie L. Devine (Scientific Associate)
Donald E. Doyle (Scientific Assistant)
Thomas E. Fritz (Veterinary Pathologist)
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Jane L. Hulesch (Scientific Assistant)
Emil G. Johnson, Jr. (Engineering Assistant)
Lillian V. Kaspar (Scientific Assistant)
William G. Keenan (Scientific Associate)
Louise S. Lombard (Veterinary Pathologist)
V. Ann Ludeman (Scientific Assistant)
+Patrick H. Polk (Scientific Assistant)

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*Transferred to Environmental Impact Studies Division in 1979.
+Terminated during 1979.

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John F. Thomson (Senior Biologist)
David V. Tolle (Scientific Associate)
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TEMPORARY STAFF DURING 1979

Donna M. Buchholz (Postdoctoral Appointee)

*Transferred to Environmental Impact Studies Division in 1979.

DOE: Carcinogenesis (HA-02-02-01)
ANL-60300

Neutron and Gamma-Ray Toxicity Studies

J. F. Thomson, D. Grahn, L. S. Lombard, F. S. Williamson, K. H. Allen, G. L. Holmblad, J. L. Hulesch, E. G. Johnson, V. A. Ludeman, A. Sallese, E. Staffeldt, and J. E. Trier

The purpose of the program is to obtain information on the late effects of low doses of ionizing radiation in laboratory rodents that can provide guidance for the prediction of radiation hazards to man. This information takes the form of dose-response relationships for life shortening from all causes and from specific causes of death.

METHODS

The program employs two radiation qualities, 0.85 MeV neutrons from the JANUS reactor and ^{60}Co gamma rays, delivered as single, fractionated, or weekly duration of life exposures, covering the following range of doses and dose rates:

	Neutrons	γ-rays
Single exposures (rads)	1-240	22.5-788
Fractionated, 2-60 fractions (rads)	10-320	206-3820
Duration of life (rads/fraction)	0.67-2.67	7-32

In most experiments we have employed the mouse (Mus musculus), specifically the C57BL/6 x BALB/c F_1 hybrid ($B6CF_1$). To provide an interspecies comparison, we have used the white-footed mouse (Peromyscus leucopus); these animals are the same size as Mus, but live about twice as long and display a distinctly different spectrum of tumors.

Animals are observed for their lifetimes; gross and microscopic examinations of the decedents are carried out to establish the probable cause of death.

SUMMARY OF RESULTS

Exposures to single doses of neutrons below 20 rads and gamma rays below 90 rads have been carried out, but too few animals have died to permit even a provisional estimate of life shortening. The following conclusions are based on life shortening after doses upward of 20 neutron or 90 gamma rads:

Fractionation of gamma irradiation results in a longer mean survival time than is observed after the same total dose is given in a single exposure; the more extensively the dose is fractionated or protracted, the less effective is the total dose.

Fractionation of neutron irradiation, on the other hand, decreases the mean survival time relative to that observed after single doses. The difference is statistically significant at doses above 100 rads; at lower doses, although the evidence for augmentation may not be convincing, there is at least full additivity.

Females are more sensitive than males to single or fractionated exposures to neutron radiation, but not to gamma radiation, principally because of a differential sensitivity to induction of lymphoreticular tumors; life shortening attributable to these tumors is 20 to 40% greater in females after exposure to neutrons, but is about the same for both sexes after gamma irradiation.

The apparent frequency of deaths caused by tumors decreases with increasing single doses of either neutrons and gamma rays; however, the age-adjusted incidence for all tumors and for numerous specific tumors shows a dose-dependent increase.

Fractionated neutron exposures are more effective than fractionated gamma exposures for the induction of lung tumors and other epithelial tumors, but significantly less effective for the induction of lymphoreticular tumors.

Gastrointestinal tumors, which are rarely seen in control mice (< 1% incidence), are a prominent cause of death (> 20%) after high single doses of neutron radiation; this increased incidence is not observed following gamma irradiation or fractionated neutron irradiation.

The radiobiological effectiveness (RBE) for single doses of neutron radiation relative to gamma radiation varies inversely with the square root of the neutron dose. Extrapolation from present data suggests an RBE of 35 at 1 rad, based on life shortening from all causes; this value is essentially confirmed for life shortening from specific tumors.

For weekly duration-of-life exposures, an RBE of 50 is estimated for a total accumulated neutron dose of 1 rad or a weekly dose of 0.08 rad per fraction. However, gamma exposures given once a week are more damaging than the same amount of radiation delivered as truly continuous radiation, and when the dose-response curve for fractionated neutron radiation is compared with that established several years ago from this laboratory for continuous gamma irradiation, the RBE approaches 100.

CONCLUSION

Data developed from this progam will enable the construction of dose-response curves for life shortening in two rodent species for all causes and numerous specific causes of death, for both single and fractionated (including duration-of-life) exposures to both high-LET and low-LET radiation.

In the case of fractionated exposures, we shall have obtained information on the influence of total dose, dose per fraction, number of fractions, and interval between fractions, which, together with the considerable body of data already obtained in this and other laboratories, will permit a thorough characterization of the radiation response.

Perhaps the single most important achievement will be the establishment of the shape of the dose-response curve (and hence the RBE-dose relationship) for somatic effects produced in whole animals by high-LET radiation down to a dose that is only a small factor greater than the lifetime background exposure of control animals.

Radiation Toxicity in Dogs

T. E. Fritz, L. S. Lombard, C. M. Poole, T. M. Seed, D. Tolle,
J. M. Angerman, S. M. Cullen, D. Doyle, L. V. Kaspar, W. G. Keenan,
P. H. Polk, and M. M. Sanderson

The goal of this program is measurement of the late effects of low doses of ionizing radiation in a large, long-lived animal, the dog, to aid in assessing hazards and understanding mechanisms of radiation damage in man. These studies address two basic problems associated with exposure to low doses of ionizing radiation:

1) obtaining reliable data on biological responses at low dose rates when exposures are protracted, and

2) extrapolation of data from experimental animals to man.

The studies use beagles to test whether the results from studies with shorter-lived, smaller, and more radiation resistant rodents, do, in fact, establish a constant radiation injury parameter, at low doses and dose rates, that is characteristic for all species. The data from these studies will help determine whether species differences in sensitivity (the dog is 3 times as sensitive as the mouse at high doses and dose rates) are a consideration in extrapolation to man.

METHODS

Young adult beagles are given whole-body exposures to protracted irradiation (22 hours/day, 7 days/week) from ^{60}Co gamma ray sources. They are exposed (1) until they die or (2) until they accumulate predetermined total doses of irradiation.

The dogs are monitored regularly by clinical, hematological, and pathological examinations. End points determined are times to death (life shortening), causes of death, and characterization of all pathological processes. Monitoring of the hematopoietic system is emphasized because of the importance of myelogenous leukemia and related myeloproliferative disorders as shown by data in man and other experimental species.

Earlier exposures, given continuously until death or terminated at predetermined total doses, used rates down to 5 R/day. In the terminated experiments, the same total exposures were given at several rates to measure the relative importance of exposure rate. Present exposures are given continuously at rates of 2.5, 1.0, and 0.4 R/day; the latter should be low enough to allow a nearly normal life-span.

SUMMARY OF RESULTS

Earlier studies, at exposure rates of 5 R/day and above, demonstrated that the hematopoietic system was the principal target organ:

There were three causes of death--septicemia, aplastic anemia, and myeloproliferative disorders (MPD's), which occurred predominantly as myelogenous leukemia.

Septicemia occurred earliest, and was the only cause of death at exposure rates of 35 R/day and above.

Anemia developed at later times, at exposure rates between 5-17 R/day, whereas MPD occurred still later at the same rates.

At 5 and 10 R/day, $\sim$ 50% of each group died of MPD's.

At 5 R/day, $\sim$ 50% of the dogs were able to accommodate to the continuous irradiation and died at significantly longer times of causes unrelated to the hematopoietic system (mostly tumors).

Protracted irradiation seems uniquely effective in inducing sarcomas that are of nerve sheath origin.

On the basis of the radiation specific mortality rate, we have shown that the dog and mouse are equally sensitive to protracted irradiation at 5 R/day, but the dog is more than three times as sensitive to acute brief exposures.

Although $\sim$ 75% of the dogs given terminated exposures are still alive, several important observations can be made:

Myelogenous leukemia has occurred less frequently than in dogs irradiated continuously.

The minimum time for induction of MPD is $\sim$ 400 days, and the minimum total exposure is $\sim$ 1700 R.

MPD has not occurred as a result of terminated exposures at rates above 17 R/day.

In addition to leukemia, the numbers of malignancies seem excessive for those groups in which appreciable numbers are dead.

Interim data also suggest that the biological behavior, but probably not the total incidence, of mammary tumors is affected by terminated exposures. Thus far, 10 of 16 (63%) mammary tumors have been malignant, whereas only 3 of 14 (21%) in controls have been malignant.

Ongoing studies at 2.5, 1.0, and 0.4 R/day are extending the observations at 5 R/day and higher dose rates to determine whether the changing sensitivity of dogs compared to mice continues at lower exposure rates:

In the continuous exposures, four dogs have died to date of damage to the hematopoietic system at 2.5 R/day--one of aplastic anemia and three of leukemia.

Leukocytes and platelets show significant depression of circulating levels at $\sim$ 300 days of exposure whereas erythrocytes show changes only after 1000 days.

The total leukocyte count is independent of dose rate, but related directly to total accumulated dose.

CONCLUSION

Data from these studies will define the response of a long-lived species so that comparisons can be made to shorter-lived laboratory species, particularly mice, in order to provide a better basis for extrapolation to man. The role of dose rate in determining biological responses, especially damage to the hematopoietic system, and numbers and types of tumors will be measured. As reflected in the hematology data to date at rates of 0.4, 1.0, and 2.5 R/day, the dog is showing a biological response that is total-dose dependent. This has been shown by the radiation specific mortality in rodents but not previously demonstrated in longer-lived animals.

DOE: Carcinogenesis (HA-02-02-01)
ANL-63105

Cellular Indicators of Preclinical Phases of Leukemia

T. M. Seed, T. E. Fritz, D. M. Buchholz, C. M. Poole, D. Tolle, G. T. Chubb,* S. M. Cullen, D. Doyle, and L. V. Kaspar

The objectives of this program are to:

1) define the stages of pathological progression of myelogenous leukemia occurring in dogs undergoing continuous radiation exposure;

2) develop sensitive predictors of such pathological responses, based on early cellular events;

3) utilize cell cloning methods to clarify mechanisms of early hematopoietic dysfunctions.

METHODS

Based on previous work of the program on Radiation Toxicity in Dogs (see preceding report), which showed that myelogenous leukemia occurs with a 50% incidence in beagles exposed for protracted periods to ^{60}Co gamma radiation, we irradiate beagles continuously (i.e., 22 hours/day) at the dose rate of 10 R/day. Our experimental approach is then to assess serially the phase related changes within the granulopoietic system of the exposed animals. Assessment employs both *in vitro* and *in vivo* assays aimed at compartmentalizing the granulopoietic system. The marrow compartments presently being examined include (1) the granulocyte-monocyte "committed" stem cells (GM-CFUa), (2) granulocyte reserves, and (3) stromal elements.

SUMMARY OF RESULTS

Earlier work identified and partially characterized four preclinical stages of radiation-induced leukemia: (I) the initial radiotoxic period, (II) partial recovery, (III) subnormal hematopoietic accommodation, and (IV) cytologically defined preleukemia.

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*Electron Microscopy Center, Division of Biological and Medical Research.

The granulocyte-monocyte "committed" stem cell (GM-CFUa) compartment initially contracts for the first ∿ 200 days during irradiation (phase I) and slowly expands thereafter (phases II-III), until in late phase III marrow GM-CFUa rapidly falls as the dog enters a cytologically defined preleukemic stage (phase IV).

The clonal growth pattern of GM-CFUa changes dramatically during preclinical phases: during the early period of GM-CFUa compartmental depletion (late phase I-early phase II) the colonies are small and loosely compacted; in the expanded compartment (phase III) the colonies are larger and more dense; and in the late period (phase IV) prior to onset of overt leukemia a dense "lawn" of single cells/small clusters is observed.

Serum titers of GM-CFUa colony stimulating activity sequentially change as well, in a reciprocal fashion, with the change in concentration of peripheral blood granulocytes.

The granulocyte reserves contract for the first ∿ 200 days, slightly expand between days ∿ 200-300, and then remain stable for several hundred days until entry into phase IV when a second expansion occurs. The composite cell population of the expanded granulocyte reserves during phase IV appears aberrantly immature.

The number of clonogenically active marrow stromal elements (plaque-forming units) initially declines during phase I and subsequently increases to normal during phases II and III. Preliminary evidence suggests a decline in capacity of the irradiated stroma to stimulate in vitro colony formation of normal GM-CFUa.

CONCLUSION

Our descriptive studies of the early granulopoietic dysfunctions induced by protracted radiation exposure and culminating as myeloid leukemia will continue. Our work to date has strongly indicated that prognostic kinetic changes in granulocyte-monocyte progenitor cell function occur and can be identified early in the leukemogenic sequence.

The focus of our continued work will be to assemble a "bank" of clonal markers for preleukemic states. This should enable us to: (1) establish the full developmental sequence, based on clonal relationships of radiation induced leukemia in a large, long-lived species; and (2) enhance our ability to detect and evaluate incipient or early hematopoietic disorders.

3. Mammalian Genetics: Genetic Effects of High LET Radiations

D. Grahn, M. L. Garriott, B. H. Frystak, and C. H. Lee

The objectives of this project are to:

1) assess genetic hazards from testicular burdens of ^{239}Pu and ^{241}Am;

2) determine retention and microdistribution of these transuranic elements in the testis in order to estimate radiation dose to germinal components;

3) compare the effects of ^{239}Pu and ^{241}Am with single, weekly, and continuous ^{60}Co gamma irradiation and single and weekly fission neutron irradiation to develop a basis for estimating relative biological effectiveness (RBE);

4) develop detailed dose-response data for genetic end points of concern at low doses of neutrons and gamma rays (RBE values at doses below 10 rads of fission neutrons are uncertain, but may be 20 or greater).

METHODS

Comparatively short-term genetic end points were used, namely: the dominant lethal mutation rate in both premeiotic and postmeiotic cell stages, the frequency of abnormal sperm head morphology measured at various times after irradiation, and the frequency of reciprocal chromosome translocations induced in spermatogonia and measured at first meiotic metaphase. Male hybrid $B6CF_1$ mice, 120 days old, were used for all studies. Measures of the retention, microdistribution, and microdosimetry of plutonium in the testes were obtained by conjoint histological and autoradiographic procedures after exposure to monomeric plutonium citrate. Concurrently, other males were exposed to external fission neutron and ^{60}Co gamma irradiation. Breeding tests were performed with the irradiated males at selected periods to obtain data on postmeiotic, meiotic, and premeiotic cell stages in male gemetogenesis.

The principal radiation exposure variables were:

	Rads/Minute (Nominal)		
	Single	Weekly	Continuous
^{60}Co gamma rays	35	2	0.003
∿0.8 MeV neutrons	5	0.01	-
5 MeV alpha particles	-	-	0.001

A limited study has also been done with a single dose of ^{241}Am.

Single neutron doses of 1, 2.5, 5, 10, 20, and 40 rads have been compared with the effects of 22.5, 45, and 145 rads of ^{60}Co gamma rays, using the same genetic end points employed in the transuranic studies. A weekly, duration-of-life exposure series has also been initiated for additional neutron:gamma ray comparisons at low weekly increments of 0.67, 1.67, and 2.67 neutron rads vs. 6.95, 17.4, and 32.0 rads of gamma rays. Males will be periodically screened for accumulating dominant lethal mutations and for the frequency of chromosome aberrations induced in the stem cell population. The objective is to correlate the rates of accumulation of dominant lethal and chromosomal damage, since the low dose studies have given positive evidence of a dose dependent accumulation of dominant lethal mutations induced in the spermatogonial population.

The sister chromatid exchange technique is being perfected for use in the neutron and gamma ray comparison studies and for future experiments with chemical mutagens.

SUMMARY OF RESULTS

Genetic Effects of Gonadal Burdens of the Transuranic Elements

^{239}Pu is deposited in the interstitial tissue of the testis and is retained indefinitely at a level of 0.053 ± 0.001% of the injected dose.

Significant Pu deposition occurs along the basement membrane of the spermatogenic tubule, thus delivering a dose to spermatogonia that is 2-4 times greater than the integrated dose to the whole organ. The extreme heterogeneity of plutonium deposition, however, leaves about 85% of the tissue radiation free.

Comparisons of ^{239}Pu with neutrons and gamma rays suggest that the alpha particle has approximately the same mutagenic effect (based on dominant lethal mutations in postspermatogonial stages) as the external fission neutron and a 10 to 15-fold greater effectiveness than ^{60}Co gamma rays. Most lethal mutations induced by plutonium are reasoned to have been induced in postspermatogonial stages.

For reciprocal translocations induced in spermatogonia, chromatid fragments induced in primary spermatocytes, and abnormal sperm morphology induced in late spermatogonia and early spermatocytes, alpha particles are 1 to 2 times as effective as weekly fractions of neutrons, but they are 25 to 40 times as effective as low intensity continuous gamma irradiation.

^{241}Am has a lower retention value than ^{239}Pu (0.031% vs. 0.053%) in the testes and its significantly different microdistribution results in lower spermatogonial exposure. Mutation and translocation rates are much below levels predicted from organ retention values, confirming the lower exposure levels for stem cells and early spermatocyte stages.

Genetic Effects of the External Radiations

The meiotic and postmeiotic (postspermatogonial) cell stages are 10 to 20 times more sensitive to the induction of dominant lethal mutations than are the stem cells. There is a statistically significant dose-dependent response of the stem cells for the induction of dominant lethals, which has not been seen previously.

The rate of induction of translocations by gamma rays is significantly reduced by fractionation and reduced dose rate, as noted by others, whereas weekly fractions of neutrons are more mutagenic than a single exposure. The response to weekly neutron exposures is linear after 10 or more exposures, is slightly concave downward after 6 to 9 fractions, and is markedly nonlinear after a single exposure.

Translocations occur at twice the frequency of lethal mutations induced in spermatogonia. This accords with expectation.

In the extremely low dose studies, there is a continuous, essentially linear response for the induction of dominant lethal mutations in postspermatogonial stages down to the lowest doses. There is a slight but not significant response in excess of predicted levels, which is not greater than a factor of 2 above expectation. The RBE at 1 to 5 rads of neutrons is no greater than 10.

For translocations, there is also the hint of response in excess of expectation for neutrons, and the RBE at low single doses would be between 5 and 10. In comparison to low intensity continuous gamma irradiation, the RBE is no less than 35 and may be greater than 50.

CONCLUSION

Studies with the transuranic elements plutonium and americium have been essentially completed. These have demonstrated that the alpha emitters have about the same mutagenic efficiency as fission neutrons and a 10- to 15-fold greater effectiveness than gamma rays. The extremely heterogeneous deposition pattern of the transuranics in the male gonad acts to reduce their biological effectiveness in terms of "dose to the gonad." This is especially evident in the induction of chromosomal damage to stem cells when measured over the reproductive life after a single injected dose has been administered. The response does not continue to rise with steadily increasing accumulated dose as might be predicted but, rather, the response remains at a steady level slightly above the control level.

Statistically significant evidence of genetic damage has been measured at neutron doses as low as 2 rads and gamma-ray doses of 22.5 rads. There was a twofold greater dominant lethal frequency at 1 and 2 neutron rads than was linearly predicted from higher dose levels (5 to 40 rads). However, this does not appear to be related to any particular cell stage in gametogenesis. The neturon/gamma-ray RBE for low single doses is generally 5 and may reach but not exceed 10 for genetic damage induced in maturing germ cell stages. Translocations induced in stem cells have the most sensitive variations in yield as exposure conditions change, so that the neutron RBE for single doses may be only 3 to 4, but shift to 30 to 40 for comparisons involving low intensity continuous gamma irradiation.

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Barbara H. Frystak (Scientific Assistant)
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Chung Hee Lee (Scientific Assistant)

TEMPORARY STAFF DURING 1979

Michael L. Garriott (Postdoctoral Appointee)

DOE: Carcinogenesis (HA-02-02-01)
ANL-60404, 60405, 60407, and
62200

4. Toxicology Program for Coal Combustion and Conversion Effluents

C. A. Reilly, Jr.

Efforts of this program are focused on the determination of the toxicities associated with a variety of developing coal combustion and conversion technologies. This program is an integral part of a much larger interdivisional program involving the Chemical Engineering Division and the Environmental and Energy Systems Division, but only those studies in which the majority of effort is within the Division of Biological and Medical Research are presented here.

Most of the effort during 1979 was directed toward studies on fluidized bed combustion of coal. These studies are reported as two sections, the first dealing with a general evaluation of the toxicity, utilizing a variety of cell test systems, and the second dealing with inhalation effects in mice. A third section covers studies on high-BTU coal gasification.

REGULAR STAFF

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Tatsuo Matsushita (Geneticist)
‡William P. Norris (Biochemist)

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*Transferred to Environmental Impact Studies Division in 1979.

†Terminated during 1979. Present address, Biology Division, Oak Ridge National Laboratory.

‡Retired in 1979.

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Donna M. Buchholz (Postdoctoral Appointee)
Frederick R. Kirchner (Postdoctoral Appointee)
George R. Lankas (Postdoctoral Appointee)

DOE: Carcinogenesis (HA-02-02-01)
ANL-60405

Comparative Biological Evaluation of Effluents from an Experimental Pressurized Fluidized Bed Combustor

D. A. Haugen, M. M. Elkind, A. Han, H. E. Kubitschek, T. Matsushita, W. P. Norris, G. R. Lankas, S. Bourne,* H. R. Isaacson,+ A. Jirka,* K. Suhrbier, M. A. Shotola, and D. M. Williams

Biological effects of organic materials found in the off-gas of a bench-scale fluidized bed combustor (FBC) were determined in a variety of in vitro cell test systems. The study was designed not only to determine whether toxic materials were present but to demonstrate the necessity for a battery of test procedures. The materials were obtained from the experimental pressurized FBC operated in the Chemical Engineering Division.

METHODS

A high volume sampler equipped with glass fiber filters was used to collect effluent from an exhaust duct at a point downstream from the final particulate filter and pressure relief valve which are part of this FBC. At the collection point, the off-gas had cooled to 37-43°C, and had been diluted 5- to 10-fold with ambient air. During each of several 1 to 2 hour collections, 0.3 to 2.4 grams of material was obtained which consisted of a mixture of variable proportions of particulate effluent and a yellow-orange, oily condensed phase. These mixtures are referred to as "condensates."

To prepare samples for biological evaluation, the filters were extracted with dichloromethane, the solvent was evaporated, and the residues (0.03 to 0.45 gram) were dissolved in small volumes of tetrahydrofuran or dimethyl sulfoxide. Filters containing no effluent were similarly treated, as controls.

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In the study described here, 10 samples were obtained during five different FBC operating periods. Preliminary testing with the Ames assay indicated about a 4-fold range in the specific mutagenicity of the extracts. Based on their relative mutagenicity, the 10 extracts were pooled to form three composite samples which were subsequently used for the comparative study. Pooling the samples provided sufficient quantities for biological evaluation by several investigators, as well as for chemical fractionation and analysis.

The biological end points employed were: mutagenesis in the Ames Salmonella reversion assay; cytotoxicity in the presence and absence of simulated sunlight, mutagenesis, and transformation in V79 Chinese hamster cells or mouse embryo-derived C3H/10T1/2 cells; and growth inhibition and sister chromatid exchange in mouse myeloma cells in suspension culture. These different testing systems are described in more detail in accompanying sections of this report.

SUMMARY OF RESULTS

The biological results are given as a composite of the three samples, since their toxicologic effects differed only by 2- to 3-fold or less.

Mutagenesis. The condensates were directly mutagenic for both S. typhimurium TA98 and TA1538. The specific mutagenicity was 10 to 20 revertants/μg. The activity was stable during storage for several months at -20°C.

V79 cells. The condensates were cytotoxic. Cell survival was 20% when the condensates were present at concentrations of 20 to 40 μg/ml for 24 hours. The presence of the condensates enhanced cell killing due to irradiation with simulated sunlight (principally near ultraviolet light).

Mouse myeloma cells. Exposure to condensate at concentrations of 10 to 20 μg/ml for 2 to 3 days caused 50% inhibition of growth. Increased sister chromatid exchange occurred following exposure to the condensates.

These biological results demonstrate that the condensates contain materials that are relatively potent in producing cellular damage as detected by several end points. Results are quantitatively consistent for the different assays in that there was a relatively narrow range of activities for the three pooled condensates in each test system.

Although there is a positive correlation between mutagenesis in the Ames assay and growth inhibition and lethality in mouse myeloma cells, preliminary data indicate no correlation or a negative correlation between mutagenesis in the Ames assay and lethality in V79 cells. These results indicate the need for several kinds of testing systems for thorough toxicologic investigation of these effluents. Interpretation of the data with regard to risk assessment of fluidized bed combustion can be only tentative because the off-gas stream in the experimental unit may not be representative of that in larger units.

CONCLUSION

Biological effects of organic materials produced in an experimental bench-scale fluidized bed coal combustor were examined in several cellular systems. The end points used were mutagenesis in bacteria, and cytotoxicity, sister chromatid exchange, and alteration of sensitivity to ultraviolet light-dependent cytotoxicity in mammalian cells. While all systems were sensitive to the materials, the range of biological effects observed indicates the need for a variety of procedures for evaluation of these complex materials.

DOE: Carcinogenesis (HA-02-02-01)
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Effects of Inhalation Exposure of Mice to Fluidized Bed Combustion Effluents

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Fluidized bed combustion of coal is a process in which ground coal and limestone are burned in a chamber in which air is forced up through the bottom giving the mixture a boiling or "fluidized" consistency. The technology is particularly adaptable to the burning of high sulfur coal. As the coal burns and releases sulfur, the calcium in the limestone chemically binds the sulfur and prevents it from escaping with the exhaust effluent as sulfur dioxide. The combustion temperature is maintained at about 850°C. This combustion temperature does not allow complete combustion of all the hydrocarbons from the coal, and the fly ash particles do not fuse into spheres. This results in particulates with very large surface areas which can adsorb onto their surface a relatively large amount of unburnt vapor phase hydrocarbon. These incompletely combusted hydrocarbon-coated particulates are capable of depositing in the deep lung spaces in locally high concentrations, and present a unique potential health hazard.

METHODS

Small rodents (mice and rats) were exposed to the effluents from the bench-scale atmospheric pressure fluidized bed combustor (FBC) in the Chemical Engineering Division (CEN) in two ways. The first exposure system, in CEN, was designed to expose the test animals to the diluted effluent on line, with the FBC as the effluent source. Two experiments were run. Experiment I consisted of a 1000-hour total exposure (divided into two 500-hour segments, separated by approximately 1 week) of B6CF$_1$ female mice to the whole effluent diluted 20-fold with air. Experiment II consisted of a similar

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exposure in which $B6CF_1$ mice and Fischer F-344 rats were exposed only to the gaseous components. The second exposure system, assembled in this division, was used to expose the animals to reaerosolized fly ash generated by a TSI fluidized bed aerosol generator (Experiment III). The fly ash had been collected from the final filter during steady-state operation of the same FBC used in Experiment I and the experiment was run under the same exposure schedule used in Experiment I.

SUMMARY OF RESULTS

The body weights of the exposed animals in all experiments were all below control levels. The only significant histological changes occurred in the lungs, and only in Experiments I and III, in which particulates were present. The changes consisted of alveolar and bronchiolar hyperplasia, followed by later recovery and repression of the changes. All other organs were normal.

An alveolar macrophage function test indicated that during the first 500 hours of Experiment I the functional ability of the pulmonary macrophage was reduced below control levels. All other test periods were in the normal range. Femoral bone marrow stem cell colony forming units were decreased in exposed animals in Experiment I and normal in all others.

Exposure to the complete effluent increased the biphenyl hydroxylase activity by a small but significant amount. Increases in hepatic monooxygenase activity have been observed by other investigators after pulmonary exposure to other agents, e.g., cigarette smoke. The data suggest that inhalation of the effluent resulted in systemic absorption of organic materials that were responsible for alteration of the hepatic monooxygenase system.

CONCLUSION

Exposures of mice to highly concentrated whole and fractionated effluents from an atmospheric fluidized bed combustor for relatively long periods produced a few minor biological changes, all related to the particulates in the effluent.

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ANL-60404, 60407, and 62200

Toxicological Assessment of High-BTU Coal Gasification Technology

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This project is one component of an integrated Argonne program that addresses the potential impact of high-BTU coal gasification on human health and the environment. The immediate objective is to characterize biological activity (mutagenicity, toxicity, and oncogenicity) of process streams and effluents from the HYGAS pilot plant. Two approaches to this characterization are being pursued: (1) qualitative identification of biohazardous materials and quantification of their degree of biological activity to determine process elements of potential concern, and (2) specific identification of the most hazardous constituents.

METHODS

A comprehensive, tiered screening procedure is used for biological evaluation and includes testing for mutagenesis in bacterial and mammalian cell systems and assay of mammalian toxicology.

Level one screening procedures. Mutagenic toxicity and cytotoxicity are evaluated in a qualitative fashion using a variety of rapid *in vitro* cell systems. For mutagenic toxicity, the Ames/*Salmonella* assay and sister chromatid exchange in mouse myeloma cells are examined; for cytotoxicity, assays in cultured V79 hamster cells, mouse myeloma cells, and rabbit alveolar macrophages are examined. (See Section 10, Mutagenesis; and Section 11, Mammalian Cell Biology.)

Level two procedures. Following qualitative identification of process streams or effluents of potential concern, simultaneous identification of the active moiety (chemical or chemical class) is attempted and the biological activity is quantitated. Procedures employed include a selective repeat of level one assays with the addition of an *in vitro* transformation assay (C3H/10T1/2 cells). Actual chemical identification requires iterative chemical fractionation of the complex mixtures under investigation and determination of biological activity. For this operation only the Ames/*Salmonella* assay is used.

Level three procedures. Following quantitation and/or chemical identification of biohazardous process components, whole animal studies are used to predict health effects in man more reliably. These studies include acute and chronic mammalian toxicology assays (for HYGAS samples, the assays selected determine oral and/or intraperitoneal LD_{50}'s, dermal sensitivity, and skin carcinogenicity).

SUMMARY OF RESULTS

An operational tiered procedure for toxicological assessment studies has been established.

Sampling of materials from the HYGAS pilot plant for biological activity was started with level one procedures.

Initial results indicate that, in general, the samples showed little or no activity. Some components of the low temperature reactor process oil and the recycle oil and, specifically, the higher molecular weight compounds exhibited mutagenicity.

CONCLUSION

Test procedure protocols have been adapted and validated and are now in use. With significant sampling of process streams and effluents, focused on the scalable units, realistic appraisals of the impact of the HYGAS coal gasification process on human health should be obtainable in the near future.

Health and Environmental Effects
Quantification (HA-02-06)
ANL-68100

Nuclear Regulatory Commission Funding
ANL-8M419
ANL-8M420

5. Human Health Risks Analysis: Assessment of Health Costs of Energy Related Pollutants

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The present research develops new statistical methodology, mathematical models, and data bases of relevance to the assessment of the health impacts of energy technologies, and uses these to identify, quantify, and predict adverse health effects of energy related pollutants. Efforts are in five related task areas. These are:

Task (1) Development of national data base of demographic, socioeconomic, climatic, and pollution variables which is being used in exploring the multivariate relationships associated with variation in human mortality data.

Task (2) Investigation of human birth weights as a possible "early warning" system for the effects of environmental pollution.

Task (3) Evaluation and developmemt of statistical procedures for the analysis of death rate and disease incidence data.

Task (4) Development of dose-response and demographic models useful in the prediction of the health effects of energy technologies.

Task (5) A reanalysis of the Tri-State leukemia survey data, focusing on the relationship between myelogenous leukemia risk and diagnostic X-ray exposure.

Tasks 1-4 are supported by the Department of Energy under ANL-68100. Task 4 is also supported by the Nuclear Regulatory Commission under ANL-8M419, and Task 5 is entirely supported by NRC under ANL-8M420.

METHODS

Task 1. Development of our national data base involves diverse data sources, including the U. S. Census, U. S. Weather Service, National Center for Health Statistics, and the City-County Data Book. Relevant data from each of these has been merged into a national county level data base which includes mortality data, socioeconomic factors, climatic variables, and air pollution data. Analysis of these data have, to date, focused on the use of regression methods to examine the influence of socioeconomic, environmental, and air pollution factors on mortality paterns.

Task 2. Our birth weight studies have used analysis of variance techniques to examine the effects of mother's age, parity (birth order), sex of child, and season of the year on mean birth weight. Two data sets have been examined, a 50% sample of all U. S. white births for the year 1970 and all white births for the Chicago Standard Metropolitan Statistical Area in 1970-1972. In addition, computer generated maps are being produced to enable us to examine spatial patterns in mean birth weight that might be related to environmental pollution.

Task 3. Our statistical studies are concerned with methodologies used in stepwise regression procedures and with a class of tests called "Poisson Trial" tests which can be used as alternatives to 2 x 2 contingency table analysis. Investigations of such properties as power and bias have been performed using both classical analytic approaches and Monte Carlo simulation.

Task 4. Our studies of dose response have focused on development of metabolically realistic models for heavy metals. These studies began with the determination of functional relationships for heavy metal dose-response relationships using standard statistical methodologies. Current efforts are devoted to refining these models through development of metabolic compartment models that will enable us to examine possible effects of individual variability.

Our health effects projection programs are based on the Leslie population projection procedures commonly used by demographers and population biologists, together with various dose-response functions for environmental pollutants.

Finally, our heavy metal dose-response functions and population projection methodologies are being used in the projection of the health effects of battery technologies. (See Section 6.)

Task 5. Reanalysis of the Tri-State leukemia survey adult data has focused on the classical measures of relative risk (Mantel-Hanzel; Wolf-Haldane procedures) to determine whether excess risks shown by earlier studies do, in fact, indicate an adverse effect of diagnostic X-rays. Studies under way will also examine the distribution of X-ray dose over time before diagnosis of leukemia and possible confounding factors, such as occupation, in the association between X-rays and leukemia.

SUMMARY OF RESULTS

Task 1. Work toward a national data base has led to production of a major review (in press) of factors influencing mortality patterns. This has proved useful as an internal reference document and should aid future investigations of mortality patterns by others.

Regression analyses of a 191 county subset of our national data set have shown that relationships between mortality rates and socioeconomic and environmental variables are not consistent among regions of the United States, and suggest that further analysis should be carried out on a regional basis.

Task 2. Analyses of human birth weights have shown that there are highly consistent effects of mother's age, parity, child's sex, and season of the year on population mean birth weight. This fact, together with an examination of county mean birth weights in the northeastern United States for the years 1970-1973, suggests that population mean birth weight may be a useful indicator of environmental stress.

Task 3. Examination of classical stepwise regression procedures has shown that, for data sets with a large number of potential independent (predictor) variables, such methods may give relatively large multiple correlation coefficients, indicating significant association between independent variables, in the absence of any real association.

Our investigation of Poisson trials testing methods resulted in a paper describing the application of these procedures to cause of death data. Related work on power functions of exact tests (binomial, Poisson) has resulted in development of a set of rules for planning experiments so that the power of such exact tests is maximized.

Task 4. Studies of dose-response and population projection methodologies have contributed to the health effects assessment portions of a number of energy technology assessment documents.

These population projection studies have also led to development of DEMPAK, a comprehensive set of computer programs for population projection and life-table calculation.

Task 5. Our analyses of the original Tri-State leukemia survey data have shown that excess leukemia risk is apparently restricted to males over age 45 exposed to 40 or more trunk X-rays. We also showed that at least one earlier analysis of the adult data made the error of including a small number of persons with therapeutic X-ray exposures; the impact of this error on the overall risk estimates appears small.

CONCLUSION

Each of the areas described above forms an ongoing part of our program in human health risks analysis. Present concerns include evaluating the applicability of multivariate statistical procedures, such as canonical correlation and multiple group discriminant analysis, for elucidating patterns in health/environmental data sets (Tasks 1 and 3), further analysis of both our birth weight and Tri-State leukemia survey data sets (Tasks 2 and 5), and expansion of our dose-response models to a broader range of pollutants (Task 4). Ultimately, these diverse but related lines of inquiry will provide a unified body of fact and theory upon which persons charged with the assessment of health effects may base their analyses.

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Health and Environmental Effects
Quantification (HA-02-02-03)
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Nuclear Regulatory Commission Funding
ANL-8M447

6. Toxicity of Systems for Energy Generation and Storage

M. H. Bhattacharyya

The overall objective of this program is to identify and investigate potential adverse health effects associated with various means of energy production and storage. The program currently includes two subtasks:

A) Assessment of the health and environmental effects of electric storage systems.

B) Metabolism and toxicity of metal compounds associated with energy production and storage.

The first subtask is an assessment program which was initiated in early 1978 and is being carried out jointly by the Divisions of Biological and Medical Research (BIM) and Environmental Impact Studies (EIS). The second subtask is an experimental research program funded partly by DOE and partly by the Nuclear Regulatory Commission (NRC). The assessment and research subtasks form a coherent unit with overlapping areas of interest and approach. The experimental program is based in part on research needs identified in the assessment subtask. The DOE and NRC research projects interrelate in their study of the gastrointestinal absorption of heavy metal ions. A description of Subtasks A and B above is provided in detail in the following sections.

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Health and Environmental Effects
Quantification (HA-02-02-03)
ANL-61210

Subtask A. Assessment of Health and Environmental Effects of Electric Storage Systems

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The purpose of this program is to identify issues and risks related to human health and the environment that are anticipated to result from production and use of electric storage battery systems. Commercialization of battery systems is likely for use in electric vehicles, utilities load leveling, solar energy storage, and energy conservation. The program is managed jointly by the Division of Biological and Medical Research and the Environmental Impact Studies Division. The project leader is Dr. R. K. Sharma of EIS.

METHODS

Our assessment of the ecological and biomedical effects of storage battery technologies includes analysis of the complete battery cycle comprising (1) mining and milling of the necessary raw materials, (2) manufacture of the batteries and their cases and covers; (3) use of the batteries, including charge-discharge cycles; (4) recycling of spent batteries; and (5) disposal of nonrecyclable components. The technologies associated with the latter phases of battery production and use are first defined. A market penetration scenario describing anticipated battery production levels and production growth rates is derived from analyses conducted under DOE support. Gaseous, liquid, and solid emissions associated with the defined technologies are then estimated. Emissions are estimated for several specified years in the assumed market penetration scenario, as well as on a per-megawatt-installed capacity of the battery. This approach on a per unit basis makes it convenient to determine by extrapolation the total effluents for any scenario. Effluents are dispersed in the environment according to a defined environmental dispersion model. Impacts on ecological systems and human health resulting from effluent dispersion are identified based on analysis of available toxicological information.

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SUMMARY OF RESULTS

In October, 1979, a document entitled "Ecological and Biomedical Effects of Effluents from Near-Term Electric Vehicle Storage Battery Cycles" was submitted to DOE for first round review. This document covered the three near-term batteries, the lead-acid, nickel-iron, and nickel-zinc systems, specifically for use in electric vehicles. Fifty to 100% increases in the industries associated with lead-acid battery production were identified as necessary by the year 2000. Substantial increases in lead mining, milling, and primary and secondary smelting were thus anticipated, with associated effluent increases. Stibine and arsine were identified as toxic gases generated during charging of lead-acid electric vehicle batteries. Thirty to 60% increases in world output levels of nickel and cobalt were estimated for the year 2000. Thirty and 100% increases in U. S. production levels of potassium and lithium hydroxides, respectively, were projected for that year. A requirement for new industries to handle recycling of nickel and cobalt was identified. Metabolic and toxicological information on animal and human systems was presented for compounds for antimony, arsenic, cadmium, cobalt, lead, and nickel relevant to the battery industries. Dose-response information, where available, was included. In a final section of the document, existing regulatory actions and standards related to the near-term battery systems were reviewed, together with proposed standards and recommendations for anticipated constituents in the air emissions, water effluents, and solid wastes from the battery-related operations.

CONCLUSION

The above assessment document has formed a starting point for a continuing assessment program within BIM and EIS related to production and use of electric storage systems. The document continues to be updated in response both to advances in technology and to changes in assessment needs within DOE. In the current program, for example, environmental dispersion information is being combined with dose-response models to produce a human health risk estimate for several metal compounds. These preliminary risk calculations will be included in the first Health and Environmental Effects Document (HEED) on Electric Storage Systems, to be completed in FY 1980. In addition, other systems, such as the zinc-chlorine and zinc-bromine batteries, are being assessed for effects on the environment and human health as they move away from the developmental stage toward commercialization.

Systems Damage (HA-02-02-03)
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Subtask B. Metabolism and Toxicity of Metal Compounds Associated with Energy Production and Storage

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Two projects are identified in this subtask. The first involves an investigation of the effects of pregnancy and lactation on the gastrointestinal absorption, tissue deposition, and toxic effects of metal compounds involved in production and use of electric storage systems. Pregnancy and lactation are periods of increased uptake by the mother of essential minerals such as calcium and iron. Increased gastrointestinal absorption of toxic metals such as lead and cadmium (which share common uptake pathways with calcium and iron) could thus also occur, making pregnancy and lactation a critical period for the accumulation of toxic metals by the mother. The second project involves a study of the gastrointestinal absorption of plutonium and other actinide elements encountered in nuclear power production, at concentrations at or below their respective maximum permissible concentrations (MPC's) in drinking water, using high specific activity isotopes. Past studies which form the basis for the current MPC for plutonium in drinking water involve use of much higher concentrations of plutonium. In these earlier studies, precipitation of plutonium in the gastrointestinal tract appears to have limited absorption, resulting in an underestimation of the gastrointestinal absorption factor applicable to the setting of the drinking water standard for plutonium.

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METHODS

Pregnant mice are exposed via drinking water to a given metal throughout gestation and/or lactation. At various times, mice are sacrificed to determine the toxic metal content of the tissues of the dams compared to their nonpregnant controls. The influence of pregnancy and lactation on the metal's gastrointestinal absorption and pattern of tissue deposition can then be assessed. Following these metabolic studies, the influence of pregnancy and lactation on the development of known toxic effects due to exposure to toxic metals can be assessed to determine whether toxic effects appear at a lower level in animals that have experienced exposure during one or more pregnancies.

The gastrointestinal absorption of plutonium is measured in mice, rats, and dogs exposed to plutonium either via drinking water or by gavage. Plutonium concentrations are determined in liver and eviscerated carcass at 6 days (mice and rats) or 4 weeks (dogs). Administered solutions are 1×10^{-10} M in plutonium (the molar concentration at MPC for ^{239}Pu) and contain the high specific activity, gamma emitting isotope ^{237}Pu (47-day half-life) in addition to ^{239}Pu (24,000-year half-life).

SUMMARY OF RESULTS

During continuous exposure to tracer levels of cadmium for the first 17 days of pregnancy, mice took up and retained 3.3 and 3.0 times as much cadmium in the kidneys and livers, respectively, as did nonpregnant control mice. Following exposure during the first 17 days of lactation, mice retained 17.5 and 7.1 times as much cadmium in the kidneys and livers, respectively, as did nonpregnant control mice. The above increases were caused primarily by increases in the gastrointestinal absorption of cadmium and, during lactation only, an increase in water consumption.

Results from studies on the gastrointestinal absorption of plutonium differed significantly in three respects from results reported by earlier investigators:

1) The fraction, f_1, of ingested Pu(IV) that was absorbed by the mouse was 2×10^{-3} compared to the median value of 3×10^{-5} reported by the International Commission on Radiological Protection in 1972. Precipitation in the small intestine (pH about 7) of the very insoluble plutonium hydroxide probably limited absorption in the earlier studies.

2) The f_1 value for Pu(IV) in citrate medium (2.5×10^{-3}) was very similar to the value for Pu(IV) in bicarbonate solution (2.0×10^{-3}). There was thus no stimulation in absorption due to the presence of citrate.

3) The f_1 value for plutonium in the VI oxidation state (1.5×10^{-3}) did not differ significantly from the value for plutonium in the IV oxidation state (2.0×10^{-3}). The value reported for Pu(VI) by earlier investigators was 10-fold higher (1.9×10^{-2}).

CONCLUSION

Increased gastrointestinal absorption and tissue deposition of cadmium, demonstrated to occur in mice during periods of pregnancy and lactation, could result in generation of toxic responses to this metal at lower levels of dietary exposure in multiparous than in nonparous animals.

Mice and rats administered plutonium solutions that are (1) low in concentration (19^{-10} M) and (2) carefully prepared to avoid hydrolysis and polymerization absorbed a substantially greater fraction of the administered plutonium ($\sim$ 0.20%) than had been observed by earlier investigators ($\sim$ 0.003%). Plutonium in our studies was thus absorbed to a similar extent as was cadmium in nonpregnant animals ($\sim$ 0.25%).

DOE: Electric Energy Systems (AK-05-20-00)
ANL-49185

7. Biomedical Effects of Energy Transmission: Effect of Extremely Low Frequency Electric Fields on Ultradian, Circadian, and Infradian Functions in Rodents

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Our objectives are to ascertain biological hazards near and about power lines and electrical switch gear, through the use of highly sensitive test systems in novel and relevant protocols. The protocols are directed at the measurement of circadian and intradiel functions including behavioral and metabolic end points and signatures that validly characterize a norm of health. These signatures are responsive to many potentially hazardous environmental disturbances that are ordinarily neglected in studies relying exclusively upon acute toxicity and mutagenicity as end points.

METHODS

Exposure facilities have been designed to enable exposure of small rodents to very well-defined electric fields in the range from 10-240 Hz, with an initial focus on 60 Hz, and at field strengths as high as 100 kV/m. The facilities permit continuous long-term observations *in situ*, and data acquisition before, during, and after field exposure. Two sets of exposure facilities have been designed, one with the electric field horizontal, the other with the field vertical to the earth.

White rats (Charles River) and mice (several genetically distinct lines of *Peromyscus* and *Mus*) are the experimental subjects. The rat facility (horizontal field), when completed for 60-Hz exposures, will consist of 20 cage units that incorporate features to measure and record continually and automatically core temperature, activity, body weight and weight gain, and the number of times and circadian phases in which a bar is pressed to gain a food reward in a behavior paradigm. The mouse facility (vertical field) will consist of eight entirely enclosed cage units that permit the monitoring of oxygen consumption, carbon dioxide production, motor activity, and telemetered body temperature (the metabolism, activity, and temperature or "MAT"

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variables). The rat facility is dedicated mainly to the search for circadian and infradian correlations with exposure, and the mouse (or MAT) facility is dedicated mainly to the search for ultradian and intradiel effects.

SUMMARY OF RESULTS

In each facility, extensive mappings of electric field distributions within the cages have been made with a modified design of a National Bureau of Standards miniprobe. The miniprobe method has been validated by comparisons within one of our mouse chambers with the difficult but classical electrolytic tank method.

In the rat facility, ten cage and exposure units have been constructed, and another ten are nearly ready for occupancy. In the completed units, nine series of field exposures have been made to date. In one series, young rats (less than 5 months of age) exposed intermittently to 67 kV/m (0.5 hours ON, 0.5 OFF) showed hyperactivity during exposure when exposure happened during the early inactive phase of the circadian cycle, but not otherwise, and there was no after effect on circadian phase during free-run. Older animals (over 5 months of age) showed no such response even at 100 kV/m. In the remaining series, a wide variety of intermittent and continuous exposures from 8 kV/m to 100 kV/m showed no ultradian responses or circadian after effects (phase shift, period alteration, dyschronism).

Eight mouse modules are fully operational, providing for exposure at field strengths up to 100 kV/m, with continuous monitoring of the MAT variables, together with videotape recording of mouse behavior under exposure. An initial experiment revealed an habituation phenomenon, in which mice showed less response on successive applications of the field. At high field strengths, preliminary studies showed that mice are aroused when fields are applied, but the degree of arousal decreased rapidly for successive turn-ons.

CONCLUSION

Two principal results have been observed so far: at very high field strengths (65-100 kV/m) hyperactivity in rats and precocious arousal in mice have been obseved in the presence of 60-Hz electric fields during the inactive phase of the circadian cycle. In the very near future, the frequency range under study will be broadened to 10-240 Hz, with particular attention to the question of whether or not any long-term (circadian, infradian, or neurophysiological) changes can be induced above and beyond the relatively mild ones that have been obseved to date.

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8. Environmental Physiology: Effects of Energy-Related Pollutants on Daily Cycles of Energy Metabolism, Motor Activity, and Thermoregulation

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This project has the following objectives:

1) Characterize the genotype-environment interactions that govern length of life, disease susceptibility, and response to environmental stress.

2) Application of methodology developed in (1) to evaluate the effects of specific pollutants (neutrons and gamma rays, coal combustion and conversion effluents, high-voltage fields, battery-related metals) on four physiological variables related to energy metabolism and thermoregulation.

METHODS

Two major instrumentalities were developed for these studies: (1) defined rodent populations for within-species and between-species genetic analysis of the interactions between organism and environment; and (2) instrumentation for continuous monitoring of four physiological variables to assess the energy metabolism and thermoregulation of the animal.

Two animal models are used for comparative genetic analysis. For analysis within species we utilize a complete diallel design consisting of the progeny of the 25 mating combinations of five widely used inbred laboratory mouse genotypes: A/Jax, BALB/c, C57BL/6, C3H, and DBA/1. This model system has been thoroughly defined in terms of survival characteristics, organ and body measurements, metabolic rate, and response to two environmental factors, chronic gamma ray exposure and lifetime exposure to low ambient temperature.

*Resident Student Associate, University of Chicago.

The second model system consists of two rodent species, Peromyscus leucopus and Mus musculus. P. leucopus lives twice as long as M. musculus, and we have already shown that there is a comparable difference in the rates of DNA repair in the two species. P. leucopus is being used in the JANUS program to assess the effects of low-level fast neutron and gamma ray exposure on a long-lived species (see Section 2, External Radiation Toxicity).

The experimental approach is to assess the energy metabolism and thermoregulation of individual mice by measuring four physiological variables: oxygen consumption, carbon dioxide production, motor activity, and deep body temperature. These variables can be monitored continuously for up to 10 days, or the system can be adapted to measure the rapid response to an acute stress. The system has been used to characterize the injury produced by ionizing radiations and coal combustion effluents.

SUMMARY OF RESULTS

Correlation of mean life-spans of 21 diallel genotypes with metabolic rate and body weight has given the first evidence of the dependence of longevity on metabolic rate within species.

The 24-hour (diel) cycle of activity and rest deteriorates with age in a dramatic way. In a group of 77 mice for which the diel cycles were measured at comparable ages, the mice with strong diel cycles proved to be longer lived than the mice with weak cycles. In other words, the more energy that the animal has available for his daily activities the longer he lives.

Exposure of young mice to ionizing radiations gives rise to permanent deterioration of the diel cycle, less efficient thermoregulation, and reduced capacity to respond to acute cold stress.

Mice exposed to the effluents of the Argonne atmospheric pressure experimental fluidized bed combustion unit showed residual damage to their ability to respond to cold stress 12 weeks after the termination of exposure, at a time when histology and clinical chemistry tests showed little or nothing amiss.

A factorial analysis of the constitutional factors in mouse longevity has identified three independent factors.

CONCLUSION

The above findings are important for the development of a rational toxicology of the long-term effects of environmental pollutants, because:

1) genetically determined individual differences are important for chemical pollutants, so the one genotype experiment design is unreliable;

2) constitutional factors of body build and activity level are in part environmentally determined and must be dealt with by means of multi-genotype, multienvironment experiment designs that permit a factorial analysis of the significant variables; and

3) the physiological measures we have developed are of proven worth as detectors of performance deficits.

REGULAR STAFF

Peter H. Duffy (Scientific Assistant)
John J. Russell (Scientific Associate)
George A. Sacher (Senior Biologist)

TEMPORARY STAFF DURING 1979

Richard S. Rosenberg (Postdoctoral Appointee)

9. Molecular Biophysics: Detection and Characterization of Damage in Molecular, Cellular, and Physiological Systems

S. S. Danyluk

The principal objectives of this basic research program are:

1) the development of fundamental structure/conformation information for biological molecules at all levels of organization, and

2) an elucidation of molecular events underlying circadian regulation processes.

To accomplish these goals, the program is subdivided into five interrelated subtasks and employs a variety of sophisticated synthetic, biophysical, and physiological methods. Details for each subtask are given in the following sections.

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DOE: General Life Sciences (HB-01)
ANL-61200

Subtask A. Chemical Synthesis of Nucleic Acid Derivatives

M. MacCoss, S. S. Danyluk, E. K. Ryu, S. H. Gray,* and R. S. White+

The principal objective of this program is the chemical synthesis of biologically relevant molecules. Four broad areas are being investigated:

1) Structural models for the determination and/or assignment of NMR parameters. Included here is the synthesis of isotopically substituted (^{2}H, ^{17}O, ^{15}N) derivatives.

2) Synthesis of base and sugar-modified nucleic acid and monomeric and oligomeric components.

3) Novel nucleoside analogues as potential chemotherapeutic agents. In particular, this aspect centers around the synthesis and evaluation of new types of prodrugs with increased catabolic stability and an increased capacity for targeting specifically to cellular membranes.

4) Development of new synthetic procedures as required to achieve the above-mentioned goals.

METHODS

A full repertoire of organic chemical synthetic methods is employed in this study. Heavy emphasis is placed on chromatography and electrophoresis in order to separate complex mixtures, and all new compounds are fully characterized by chromatography, elemental analysis, melting point, and spectroscopic (NMR, UV, MS, and IR) measurements. Biological evaluations of bioactive compounds are carried out in collaboration with Dr. T. Matsushita of the Mutagenesis Group in this Division (mouse myeloma MPC-11 cells) and Dr. C. I. Hong of the Roswell Park Memorial Institute (L1210 lymphoid leukemia).

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*Laboratory Graduate Program Participant, University of Illinois at the Medical Center.

+Resident Associate.

SUMMARY OF RESULTS

A complete series of nucleoside 2',5'- and 3',5'-cyclic monophosphates having ribo, arabino, xylo, and lyxo sugars, and with the aglycone oriented α or β, was synthesized. These model compounds enabled a comprehensive evaluation of intramolecular interactions involved in these rigid bicyclic ring systems and have led to the determination of quantitative torsion angle dependences for vicinal P-H and H-H coupling constants. Other conformationally invariant derivatives that were synthesized enabled a complete evaluation of the C^{13}-H vicinal correlation about the C1'-N1 bond of pyrimidine nucleosides (Subtask B).

Among selectively labeled compounds prepared were selectively deuterated NAD+ (allowing the first unequivocal assignments of the ^{31}P resonance and the H5', H5", and H4' resonances of both moieties), and uridine and 2',3'-O-isopropylideneuridine selectively enriched with ^{17}O at C2 or C4 carbonyl groups. Synthesis of the latter compounds opened up a new area of nucleic acid NMR investigation.

Base-modified nucleic acid derivatives synthesized include all of the dinucleoside monophosphates containing N1-methyladenine, the dinucleoside monophosphates containing adenine N1-oxide and cytosine N3-oxide, and heterodinucleoside monophosphates containing uracil photohydrates. These compounds were synthesized as part of a comprehensive chemical-structural study of the effects of physical and chemical mutagens on nucleic acid conformational properties.

New prodrugs of the chemotherapeutic agents arabinocytidine (araC), arabinoadenosine (araA), and tubercidin (Tu) have been synthesized in which the parent drug is attached via a 5'-pyrophosphate linkage to the membrane component 1,2-dipalmitin. Of these derivatives, araCDP-L-dipalmitin and TuDP-L-dipalmitin have been tested in *in vitro* and *in vivo* studies, and the former has shown a much increased efficacy, relative to the parent araC, against L1210 lymphoid leukemia in mice.

New synthetic procedures developed in this work include a novel modification of the Dittmer-Lester reagent for the detection of phospholipid derivatives on thin-layer chromatograms; a facile method for the simple high yield chlorination of pyrimidine and purine derivatives, some of which have not been previously accessible; the use of nucleoside N1-oxides to prevent intramolecular cyclization while carrying out nucleophilic displacement reactions on the sugar moiety of adenosine derivatives; and the use of hexachlorodisilane to deoxygenate nucleoside N1-oxides (this has led to a simple, high yield preparation of the chemotherapeutic agent 2-azaadenosine).

CONCLUSION

The main thrusts of this subtask lie essentially in two directions, namely (1) the synthesis of new biomolecules as structural probes for biological structure/function relationships (this is closely allied to

the spectroscopic goals in Subtask B) and (2) the synthesis of new analogues of biological molecules (particularly nucleoside derivatives) as potential chemotherapeutic agents. Often, these two aspects are complementary since new analogues are often excellent structural probes.

Work is continuing in the field of isotopic substitution (particularly as regards ^{17}O incorporation into cytidine and guanosine for investigation by ^{17}O NMR spectroscopy) and the synthesis of oligonucleotides having specific structural modifications in order to elucidate the conformational changes induced by such modifications. Furthermore, phospholipid-nucleoside drug conjugates are being synthesized in which the drug (araC or Tu) is linked to the phospholipid via a monophosphate linkage. It is hoped that these derivatives will show the same promise as the pyrophosphate analogues (see above) as antileukemic drugs.

Subtask B. Structural and Conformational Properties of Biological Molecules in Solution

S. S. Danyluk, M. MacCoss, H. M. Schwartz, C. F. Ainsworth, and B. Hammer*

This subtask has as its principal goals (1) the quantitative determination of structures and time-averaged conformations for biological molecules in solution, and (2) the correlation of such structural/conformational properties with mechanisms of biological function. Among molecules of primary interest are those involved in gene expression, membrane organization, and antibody function. Although emphasis has been placed upon development of base-line information for naturally occurring biomolecules, our interest has broadened more recently to include structure/conformation analyses for biomolecules chemically modified by physical and chemical mutagens.

METHODS

High-resolution ^{1}H, ^{13}C, ^{31}P, and ^{17}O nuclear magnetic resonance spectra are measured for biological molecules in solution, and the resultant spectroscopic parameters (chemical shifts, coupling constants, nuclear spin relaxation times) are then used to derive conformational features and molecular dynamics. Because of the spectral complexity of most biological molecules, extensive use is made of selective ^{2}H, ^{13}C, ^{17}O isotopic enrichment (see Subtask A), multiple resonance, and computer simulation to analyze spectra unambiguously.

In those cases where NMR signals are broadened by dipolar and/or anisotropy effects, e.g., in tissues or whole cells, a new magnetic resonance approach, i.e., cross-polarization magic angle spinning is being employed for measurement of high-resolution ^{13}C and ^{31}P spectra.

SUMMARY OF RESULTS

Extensive proton NMR measurements and conformational analyses have been completed for all pair-wise combinations of diribonucleoside monophosphates. From these studies have emerged insights into the factors governing overall

*Laboratory Graduate Program Participant, Northwestern University.

time-averaged structures and conformational dynamics for these building blocks of RNA molecules. A particularly significant finding was the existence of conformational interrelatedness among conformational bonds of the dimers.

Detailed NMR conformational analyses for base-modified diribonucleoside monophosphates have shown that methylation (A, G bases) and photohydration (U bases) produce profound changes in conformational structures relative to parent dimers. These alterations are of such a scale as to suggest a role for modified bases as structural modulators in ribonucleic acids.

A complete conformational analysis was made (in collaboration with investigators at the State University of New York, Albany) of the trinucleotide CpCpA. The results show this molecule possesses considerable conformational flexibility, a characteristic that may be required for adaptability of the CpCpA terminus to the synthetase during the amino acyl charging step.

Spectroscopic analyses were completed for a series of α- and β-arabino xylo, lyxo, cyclic nucleosides/tides encompassing all possible rigid ring combinations. The data are being used to develop empirical correlations of coupling constants, chemical shifts, and relaxation times with torsion angle variables.

^{17}O NMR spectra were measured for carbonyl oxygens of uridine nucleosides. This represents the first successful observation of ^{17}O resonances for nucleic acid derivatives and opens up a new probe for monitoring of electron delocalization and molecular interactions.

Preliminary ^{13}C cross-polarization magic angle spectra were recorded for several representative condensed state biological samples, i.e., canine tendon, polycrystalline RNA, and coal. Dramatic improvements in spectral resolution occur for these materials, opening up the possibility of high-resolution NMR for biopolymers in intact biological systems.

CONCLUSION

Work is continuing on the solution conformational properties of key biomolecules using a variety of spectroscopic techniques. In particular, we have a strong interest in nucleic acid components both in the naturally occurring state and when modified in a specific way by selected mutagens or carcinogens. Furthermore, the use of ^{17}O NMR spectroscopy (closely allied to the synthetic aspects necessary for specific enrichment--see Subtask A) to define π-bond orders, hydrogen bonding specificities, and metal binding interactions for nucleosides and nucleotides is playing a new role in the understanding of these important physical phenomena.

Finally, the recent acquisition of an NT-150 cross-polarization magic angle spectrometer now makes possible the definition of structural and conformational features of biological macromolecules in intact biological systems. Those studies promise to open new areas of biomolecular structure-function relationships in the near future.

Subtask C. Crystallographic and Chemical Studies of Immunoglobulin Structure

M. Schiffer, F. Stevens, F. A. Westholm, N. Panagiotopoulos,* and L. E. Lambert+

The objectives of this subtask are as follows:

1) To understand the function and specificity of antibodies on a molecular level.

2) To obtain precise three-dimensional structure of immunoglobulins in the crystalline state by X-ray diffraction techniques, complemented by gel filtration and small angle neutron scattering studies of antibody molecules in dilute solution. Emphasis has so far been placed upon structure determination of light chains (Bence-Jones proteins). These proteins are excellent models of intact antibody molecules.

METHODS

Data on single crystals are collected by a computer controlled diffractometer, and new crystals are characterized by precession techniques using a high intensity X-ray beam from a rotating anode X-ray generator. Small angle neutron scattering was performed at Brookhaven National Laboratory (in collaboration with Drs. B. Schoenborn and D. Carlson). Extensive use is made of the excellent Argonne central computing facilities and services. The human Bence-Jones proteins are supplied by Dr. A. Solomon of the University of Tennessee; Dr. Solomon, with whom we are collaborating, has characterized these proteins clinically and immunochemically.

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*Visiting Scientist, Nuclear Research Center "Demokritos," Athens, Greece.

+Summer 1979 participant in the Undergraduate Honors Research Program, Williams College.

SUMMARY OF RESULTS

We have characterized and purified 45 different Bence-Jones proteins, and have attempted to crystallize both their monomeric and dimeric forms. Seven proteins formed micro-crystals.

Using gel filtration, and a special computer method we worked out to simulate the behavior of associating proteins upon gel filtration, we found that the dimerization constant of κ_I light chains ranged from $< 10^3$ to $> 10^6$ M^{-1}. We identified the hypervariable region as responsible for the variation in dimerization constant: an aromatic or hydrophobic residue at hypervariable position 96 enhances dimer formation, whereas when there is a charged residue at that position the light chains remain stable monomers. These results can be extended to the formation of functional antibody molecules.

We were successful in crystallizing three new Bence-Jones proteins in a form useful for X-ray diffraction studies. Fin is the first intact κ type protein ever crystallized. Using molecular replacement methods, the orientation of the variable and constant domains in the crystal has been found. The unit cell dimensions and space group have been determined and data collection has started for λ_{IV} type protein Cle and the variable fragment of the κ_I type protein Wat.

We have determined the structure of the Mcg Bence-Jones dimer (λ type light chain) and have undertaken the crystallographic refinement with 2.3-Å resolution data. During the refinement, the crystallographic R factor decreased from 43 to 26%. The positions of 3060 of 3220 nonhydrogen atoms of the molecule are now defined. Proline 145 was identified as a cis proline, and the equivalent to Pro 145 is conserved in almost all constant region domains. Further, there is evidence that the antibody binding site is distorted by lattice forces in the Mcg Bence-Jones protein crystal.

Results from small angle neutron scattering experiments with solutions of the Mcg Bence-Jones protein show the experimentally derived scattering curve and the radius of gyration to be in agreement, within the error of the measurement, with the ones calculated on the basis of refined atomic coordinates. Therefore, the Mcg Bence-Jones protein in solution has been shown to have a structure similar to that in the crystal.

CONCLUSION

We are continuing our efforts to determine new immunoglobulin structures, and will be able to make detailed comparisons with the refined coordinates of the Mcg Bence-Jones protein. Small angle neutron scattering studies show that the information derived from crystallographic studies is relevant in dilute solution. Association properties of immunoglobulin chains can be explained by examination of their three-dimensional structures.

Subtask D. Instrument Design and Development for X-Ray and Neutron Scattering Studies of Biological Molecules

C. Borso, S. S. Danyluk, M. Schiffer, F. S. Williamson,* G. L. Holmblad,* and C. K. Eastman+

The objectives of this task are:

1) To design and develop instrumentation and software for a small angle neutron scattering (SANS) diffractometer on the intense pulsed neutron source (IPNS) for investigation of biological systems. The investigators involved in this task were C. S. Borso, S. S. Danyluk, M. Schiffer, F. S. Williamson, and G. L. Holmblad.

2) To design and develop a small angle X-ray scattering (SAXS) instrument using a self-scanning photodiode array as a linear position sensitive detector for use on conventional X-ray generators as well as synchrotron sources. The investigators involved in this task were C. S. Borso, S. S. Danyluk, and C. K. Eastman.

METHODS

The SANS diffractometer for use on the IPNS1 has been designed with the following specifications:

Flux on sample	4×10^4 neutrons/cm^2/sec
Max area of sample	3.5 cm^2
Neutrons/sec through sample	1.45×10^5
λ range	0.8-6.0 Å
Qmin	3.8×10^{-3} Å^{-1}
Qmax	2.6×10^{-2} Å^{-1}
$\Delta\theta$, angular resolution	2.0 milliradians

*External Radiation Toxicity Group.
+Student Aide.

Because small angle neutron and X-ray scattering provide complementary information, an X-ray diffractometer employing a self-scanning photodiode array detector is being developed to enhance our total capability in small angle scattering. The detector will provide a 25-micron spatial resolution and is capable of easily detecting 10^8 photons/second; it will thus be of tremendous potential for synchrotron sources.

SUMMARY OF RESULTS

SANS Diffractometer

An automated sample changer and environmental controller for use with biological samples was designed, fabricated, and assembled. The mechanism will hold up to 12 different sample vials and control the temperature to ± 2°C over 0-40°C temperature range.

A microprocessor-based data acquisition system has been implemented to transfer data from the two-dimensional detector to the Sigma 5 computer facility in the Chemistry Division.

Software routines for the reduction and analysis of small angle scattering data on ZING-P' have been written. Software from the National Center for Small Angle Scattering Research at Oak Ridge National Laboratory has been modified and implemented on the Sigma 5 computer for use at ZING-P'.

SAXS Diffractometer

Special sample cells have been fabricated to initiate preliminary investigation of rhodopsin, cytochrome c, concanavalin A, and tRNA, and software has been developed to analyze the small angle scattering data.

Analysis of the data has yielded measurements in good agreement with calculations, and excellent resolution and signal to noise ratio have been observed. Changes in the radius of gyration as a function of pH, ionic strength, and ligand binding have been measured for various proteins.

CONCLUSION

The SANS diffractometer and associated data acquisition system are in the final stages of development and waiting for checkout on the spallation neutron source. This instrument should open new areas of scientific research with high epithermal neutron fluxes, making anomalous resonance and inelastic scattering measurements feasible.

The SAXS instrument has been completed and is being routinely used for small angle studies. With its excellent resolution and tremendous count rate capabilities, it should prove to be of significant utility at existing and future national synchrotron sources. It will probably be the prototype for instruments used to initiate time resolved X-ray scattering and diffraction on high flux sources of this nature.

Subtask E. Chronobiology and Circadian Regulation

C. F. Ehret, A. L. Cahill,* N. D. Horseman,* K. R. Groh, J. C. Meinert, L. W. Ching,† M. A. Mueller,† P. Ogor,** and G. R. Schwartz††

The goal of this project is to elucidate the molecular events underlying circadian regulatory processes in mammalian and cellular systems. Emphasis is placed upon the chronobiotic action of diet, drugs, and environmental agents upon circadian phase, and the coupling of such action with neurochemical and endocrine functions.

METHODS

Automated and programmable circadian respirometers and animal environmental chambers are used for growth, entrainment, and recording of performance of ultradian and circadian cellular and organismic functions. In the mammalian studies, Charles River rats are used. The cellular studies make use of protistan and mammalian cells, including the ciliated protozoan Tetrahymena pyriformis, rat hepatocyte cell lines, and Ruber rat hepatoma cells.

SUMMARY OF RESULTS

Turnover rates of norepinephrine and dopamine in various brain regions were determined as a function of the circadian phase of administration of the specific catecholamine synthesis inhibitor α-methyl-ρ-tyrosine (α-MT),

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using high performance liquid chromatographic methods. Hepatic glycogen and tyrosine aminotransferase (TAT) levels were also measured, and TAT was found to be chronotypically inducible by α-MT.

A parallel study of the chronobiotic action of α-MT showed that it is a powerful resetter of the rat's circadian core temperature rhythm, advancing or delaying its phase as a function of the circadian phase of administration. These effects, and those described below for dexamethasone, are collectively known as a phase-response curve. Heretofore, such curves have generally been demonstrated in circadian systems only for environmental synchronizers such as light and temperature.

We showed that the widely used glucocorticosteroid analogue dexamethasone (DEX) is also a powerful circadian chronobiotic drug. As a function of the circadian phase during which injections are made, an injection of DEX either advances or delays the phase of the temperature rhythm of the rat, or has no effect. During the daily interval when endogenous corticosteroid secretions rise chronotypically, there is no effect; this interval is followed by an interval during which DEX delays the thermal rhythm, which in turn is followed by an interval when phase advances are induced.

The DEX results lead to two conclusions: (1) Therapeutic use of glucocorticoids can disturb circadian rhythms in the manner predicted from the phase response curve. (2) Endogenous corticosteroid is a significant element in the hierarchy of organismal circadian organization.

CONCLUSION

The principal results to date have shown that α-MT and dexamethasone are both chronobiotically active. Future work will include the folowing:

Studies will continue on the chronobiotic action of α-MT in rats and in rat brain, and sensitive liquid chromatographic assay methods for the rate limiting enzyme tyrosine hydroxylase, the putative α-MT target, will be applied to the catecholamine circadian turnover problem. Similar studies on the role of indoleamine metabolism in circadian regulation wll be initiated: our work to date has proved the predicted chronotypic vulnerability of the metabolic pathways associated with the active-awake phase of the circadian cycle (catecholamine metabolism), and it will be interesting to see whether similarly expected relationships hold true for the inactive-sleep phase (indoleamine metabolism) in the steps from tryptophan to serotonin. At the cellular level, the circadian respirometer will be used to see whether chronobiotically active drugs and hormones can serve as circadian synchronizers for free-living protistan and mammalian cells; of primary interest will be a study of the separate and potentially interactive roles of DEX and α-MT. This will give some clue as to mechanisms whereby circadian synchrony and circadian coordination between the tissues and organs of an intact animal are achieved and maintained.

10. Mutagenesis: Molecular and Genetic Mechanisms of Environmental Mutagens

H. E. Kubitschek, B. Hass, T. Matsushita, R. B. Webb, P. L. Derstine, V. M. Griego, M. S. Brown, S. S. Dornfeld,* J. Hibbard,+ G. Matsushita,‡ M. A. Shotola, M. A. Turner,** D. Venters, and D. M. Williams

Because mutagens and carcinogens act by producing lesions in cellular DNA molecules, this program is concerned primarily with the nature of DNA lesions produced by environmental and energy-related mutagens, their mechanisms of action, and their repair. This information is vital to the rational development of improved mutagen testing systems, to assessment of human risk, and to development of procedures for reducing mutational damage. Bacteria are used because of the large body of information and expertise available, as well as their detailed genetic maps and the many well-defined mutant strains available. Mammalian cells also are used because these have mutagen sensitivities more similar to those in man. Repair of lethal lesions induced with ultraviolet, near-ultraviolet, and visible light receives special emphasis, as human populations are exposed to large integrated doses of these radiations.

METHODS

Mutagens are chosen for study on the basis of their potential for analysis (genetic probes), for development of procedures for reducing mutational damage, for their potential importance to risk assessment, and for development of improved mutagen testing systems. Bacterial cells (Escherichia coli, Bacillus subtilis, Salmonella typhimurium) and mammalian cells (mouse myeloma mutants, Chinese hamster cells) are used; a part of this program concerns comparative studies of similar genetic lesions in both systems. The conventional tools of microbial and molecular genetics are used, along with intercomparison of genetically related strains. Advantage is taken of common techniques for studying lesions such as pyrimidine dimers and DNA strand

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‡Cytogenetic Consultant.

**Sponsored by the Argonne Affirmative Action Program.

breaks in repair-competent and repair-deficient strains. The use of continuous culture techniques often provides advantages. The techniques and advantages of continuous culture extend to studies of mouse myeloma cells maintained in suspension cultures, which have the additional advantage that chromosomal effects can also be measured by sister chromatid exchange (SCE) in conjunction with cytotoxicity assay.

SUMMARY OF RESULTS

The first evidence for coordinate induction of multiple mutations induced by UV and several other mutagens was obtained. Mutations are clustered over a genetic range of the order of 10-50 kb.

A novel approach was used to examine the effects of lesions produced in DNA by incorporated analogues, by allowing growth and segregation before production of the lesion. When cells were labeled with deoxyuridine and photolyzed at increasing intervals, survival increased from 5 to 100% after the first round of DNA replication. The method has been used to measure the reproducibility in the timing of DNA synthesis, which was found to be much more tightly controlled than cell division.

Using the above approach, it was found that survival of ^{125}I-labeled cells, inactivated to the 5% level, increased to only 50% after the first round of DNA replication. The results indicate that repair of lesions induced by ^{125}I decay is dependent upon recombination repair (*recA*$^+$) and that the repair process may be strand asymmetric.

We were also the first to demonstrate that mutation induced by near-UV (365 nm) is photoreactable, but lethality is not. These results implicate pyrimidine dimers in mutagenesis induced by near-UV light but not in cell killing by near UV.

Acridine orange plus 500-nm irradiation was shown to be far more mutagenic in recombination-deficient bacteria than in wild-type strains, and strains deficient in excision repair have even lower mutation rates. It is concluded that high mutation rates in the *recA*$^+$ strains are a consequence of semiconservative DNA replication in the presence of unrepaired lesions.

Agents that quench singlet oxygen (diazobicyclo octane, azide, histidine) were shown to protect biological material (transforming DNA, cells) against near-UV but not far-UV light. It was also discovered that glycerol protects biological material against near-UV, but not against far-UV light.

We were the first to demonstrate that near-UV induces strand breaks in DNA, both true and alkali-labile breaks.

The first action spectrum for single strand breaks produced by near-UV in DNA *in vitro* was obtained, as well as the first action spectrum for the protective action of a singlet oxygen scavenger.

A system for rapid monitoring of cell growth and cytotoxicity testing of mouse myeloma suspension cultures has been developed for screening environmental pollutants and evaluating antitumor drugs.

The fluorescence plus Giemsa method for staining harlequin chromosomes has been adapted for use in mouse myeloma cells, enabling analysis of sister chromatid exchange. Thus, both genotoxic and cytotoxic assays of drugs and pollutants can be performed simultaneously. The approach also has led to basic studies of the role of pyrimidine dimers in SCE induction during far- and near-UV irradiation, and to measurement of delay in DNA replication.

Biochemical techniques have been adapted for measuring DNA damage in mouse myeloma cells. These techniques include the use of Micrococcus luteus UV-endonuclease and Bacillus subtilis uracil glycosylase as specific probes for pyrimidine dimers and uracils. Dimer removal during cell growth indicated that partial excision of pyrimidine dimers is not a requirement for repair of far-UV induced damage in mouse myeloma cells. In Chinese hamster cells, removal of uracil was rapid and complete, and not inhibited by caffeine.

CONCLUSION

With increasing world populations, energy expenditure, and manufacturing output, there is an increasing need to understand better the nature of action of environmental pollutants, especially those produced by man, in producing mutation and cancer. Our group is making good progress in unraveling the mechanisms of action of some of these agents. Our studies and similar research of others hold the promise that biological damage may be reduced or eliminated even after individuals have been exposed and biological insults have been initiated.

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*Terminated during 1979. Present address, Oak Ridge National Laboratory.

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ANL-61302

DOE: Mutagenesis (HA-02-02-02)
ANL-61300

National Institutes of Health; Radiological Assessment of the Fermilab Neutron Beam
ANL-85903

11. Mammalian Cell Biology

M. M. Elkind

The Mammalian Cell Biology Group has, as a primary interest, the role of repair processes in the changes induced in cell properties by both agents in the environment and those used to treat cancer. The agents of interest are ionizing and nonionizing radiation; chemicals used in industry, agriculture, and medicine; and effluents of largely unknown composition produced by different energy technologies (e.g., see studies reported in Section 4, Toxicology Program for Coal Combustion and Conversion Effluents). The altered cell properties of interest are reproductive cell death, mutation, and neoplastic transformation.

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DOE: Mutagenesis (HA-02-02-02)
ANL-61300

Mechanisms of Lethality and Radiation-Induced Changes in Mammalian Cell Properties

M. M. Elkind, A. Han, E. Ben-Hur,* C. Hill,+ G. R. Lankas, H. Utsumi,+ E. Buess, J. L. Dainko, C. M. Liu, and L. D. Theriot

This program is concerned with the molecular and cell biology of radiation action in mammalian cells. Our interests are, at one extreme, to contribute to an understanding of epidemiological aspects of both ionizing and nonionizing radiation exposure and, at the other extreme, to understand the essential cell biology involved in the use of radiation to treat cancer.

METHODS

Principal methods are those pertaining to the growth and assay of cultured mammalian cells plus conventional labeling techniques and methods for measuring DNA integrity.

SUMMARY OF RESULTS

Accomplishments in four principal topics are described.

Potentially lethal damage. The treatment of Chinese hamster cells (e.g., for 20 minutes) immediately after X-irradiation with anisotonic phosphate buffered saline (PBS) significantly enhances cell killing by inhibiting repair under conditions that do not affect the viability of nonirradiated cells. The repair occurs after X-ray or neutron exposures, but not after the far-UV or the near-UV exposure of cells, whether or not they had been photosensitized by prior growth in the presence of bromodeoxyuridine.

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Repair of "single-hit" damage. In repair competent cells, the initial slope of the survival curve is increased by anisotonic PBS treatment. However, this increase does not occur in repair deficient variants in which the initial slope is steeper than that of repair competent cells without anisotonic PBS posttreatment. We conclude--contrary to commonly held dogma--that "single-hit" damage is reparable.

Repair of subtransformation damage. Using C3H mouse-derived 10T1/2 cells, X-ray dose fractionation data have been extended to low dose rate irradiation using ^{60}Co gamma rays. A dose rate range from 100 to 0.1 rads/minute was examined. The results show that subtransformation damage--i.e., damage that is subeffective--is repaired as well as sublethal damage for both small and large total doses.

The DNA action spectrum and the ozone problem. Germicidal Lamps (principally 254-nm emission, UV-C light) and filtered or unfiltered Westinghouse Sun Lamps (UV-B light) were used to measure cell killing and mutation, with V79 Chinese hamster cells, and cell killing and induction of transformation, with 10T1/2 cells. Survival data could be consistent with DNA action. However, mutation and transformation results both are inconsistent with DNA action.

One implication of the foregoing is that reductions in the earth's ozone layer will not incur increases in the rate of skin cancers to the same degree as would be predicted if the wavelength dependence completely followed a DNA action spectrum. On the other hand, the data suggest that wavelengths longer than 300 nm are more effective in inducing altered cell properties than would be predicted on the basis of DNA action alone.

CONCLUSION

The research on the foregoing cell studies with both ionizing and nonionizing radiations is being extended to an examination of molecular mechanisms. Studies of DNA integrity, synthesis, and maturation are under way.

DOE: Carcinogenesis (HA-02-02-01)
ANL-61302

New Cell Systems for the Study of the Biology of Mutation and Neoplastic Transformation

M. M. Elkind, A. Han, F. M. Buonaguro, E. Buess, J. L. Dainko, C. M. Liu, and L. D. Theriot

Fibroblasts are at present the primary cell type available for studies of the mechanism of neoplastic transformation and for screening purposes. Neoplastic transformation of these cells leads to fibrosarcomas, whereas the major neoplasms in humans are carcinomas (epithelium derived). This program is intended (1) to establish epithelial cell lines and additional fibroblast-derived cell lines for studies of mutation and neoplastic transformation, and (2) to examine the biological mechanisms responsible for phenotypic expression after transformation induction, and its relationship to phenotypic expression after mutation induction.

METHODS

Cells derived from mammary gland tissue of Wistar/Furth rats in mid-pregnancy were placed in culture, and epithelial-like cell lines were obtained by the technique of clonal isolation. Subclone G1-4 has been in continuous culture for the past 9 months. The cells were examined for their growth properties, including cloning efficiency, and their survival characteristics following exposures to graded doses of ionizing and nonionizing radiations, as well as to chemical carcinogens. The ability of untreated cells, and those treated with different carcinogens, to grow in semisolid agar was also examined.

SUMMARY OF RESULTS

G1-4 mammary cells seem to constitute an immortal line since they have been successfully grown in culture as substrate attached cells for more than 9 months. The cells exhibit a doubling time of about 17 hours, and display contact inhibition of growth with a cell density at confluency of about $5 \times 10^4/cm^2$. The plating efficiency of substrate attached cells is 40%, whereas the cloning efficiency of G1-4 cells in soft agar is less than 1×10^{-4}%. Thus, it appears that G1-4 cells are not transformed.

The survival of G1-4 cells following exposure to varying doses of 50-kVp X-rays is typical of other mammalian cells; the survival curve has a D_q of 240 rads and D_0 of 115 rads. However, the survival curve following exposure to fission-spectrum neutrons from the JANUS reactor is exponential with a D_0 of 70 rads. Exposure of G1-4 cells to UV-C (254 nm) or UV-B (290-345 nm) radiation showed a response within the range typical for most mammalian cells.

Exposure of G1-4 cells to 7,12-dimethylbenz(a)anthracene and 3-methylcholanthrene (concentrations of up to 10 µg/ml) for 24 hours results in greater than 50% survival, indicating that these cells lack the mixed function oxydase system required for metabolic activation of polycyclic aromatic hydrocarbons.

Attempts to induce measurable levels of colony formation in soft agar (i.e., transformation) following exposures to 200 and 600 rads of X-rays or 155 rads of fission-spectrum neutrons have been unsuccessful thus far.

CONCLUSION

The need to develop adequate <u>in vitro</u> systems for studying the transformation of epithelial cells is well recognized. This work will go forward using new assay conditions, such as collagen gels, to grow mammary gland derived cells and using altered morphological colonial growth as an indicator of transformation. Tumor formation in appropriately treated hosts will be used as the ultimate measure of transformation.

National Institutes of Health; Radiological Assessment of the Fermilab Neutron Beam
ANL-85903

Comparative Properties of Ionizing Radiation

M. M. Elkind, A. Han, F. M. Buonaguro, C. Hill,* E. Buess, and J. L. Dainko

The understanding of radiobiological mechanisms can be advanced by the use of ionizing radiations having different rates of linear energy transfer. X-rays and gamma rays have low rates since they deposit energy in absorbers by setting energetic electrons into motion. Neutrons lose energy mainly by interacting with protons and, because of the greater mass of the proton compared to the electron, in effect have greater rates of linear energy transfer than X-rays or gamma rays. In addition to sources of X-rays, gamma rays, and fission-spectrum neutrons at the Argonne National Laboratory, the Mammalian Cell Biology Group has the use of very energetic neutrons produced in the Cancer Therapy Facility of the Fermi National Accelerator Laboratory (Fermilab), Batavia, Illinois. This beam of particles is being used to extend studies of radiobiological mechanisms via a comparative examination of biological effects.

METHODS

Principal methods are those pertaining to the growth and assay of cultured mammalian cells plus conventional labeling techniques and methods for measuring DNA integrity.

SUMMARY OF RESULTS

Cell Killing

Because of the need to understand the radiobiological properties of the Fermilab neutron beam in connection with tumor treatment, we have measured the sparing effect of hypoxia and how this effect may vary as the average energy of the beam is degraded due to absorption in tissue. Although the sparing effect due to a lack of oxygen is about the same for Fermilab neutrons as it is for fission-spectrum neutrons, the protective effect of

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hypoxia in the former case becomes somewhat less with penetration. In respect to cell killing, the single and fractionated dose responses of Fermilab neutrons lie between those due to X-rays and to fission-spectrum neutrons, as would be expected from the comparative energy loss characteristics of these three radiation sources.

Mutation and Transformation

Consistent with cell killing studies, the efficiencies of mutation and transformation of Fermilab neutrons also appear to lie between those of X-rays and fission-spectrum neutrons.

CONCLUSION

Future measurements with the Fermilab beam are needed to extend current data on mutation and transformation not only for purely radiobiological reasons, but also because of the need to know what level of risk of new cancers may be associated with cancer therapy using this beam, and whether different modes of dose delivery might reduce the level of risk.

DOE: Carcinogenesis (HA-02-02-01)
ANL-60400, 60401, 60402, 60500

National Cancer Institute Contract
Y01 CP 7-0504
ANL8D405

12. Carcinogenesis

C. A. Reilly, Jr.

The Biological and Medical Research Division's activities in carcinogenesis represent a balanced approach between the more basic aspects of the field (mechanisms of tumor induction) and the applied aspects (screening procedures for detection of tumor initiators and promotors and their resultant tumors). Five individual projects are funded in this program. Three of these deal with mechanistic studies of hepatocarcinogenesis, the fourth explores the potential of hepatic neochromatin antigens as markers of neoplasia, and the fifth examines the role of retroviruses in radionuclide-induced osteosarcomas. Two additional studies on the microsomal monooxygenases system, funded as coal toxicology projects (ANL-60405) are reported here because of their relevance to basic mechanisms of carcinogenesis. One additional study relevant to carcinogenesis but not reported here, deals with the development of new *in vitro* mammalian cell systems for detection of carcinogens (see Section 11, Mammalian Cell Biology).

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DOE: Carcinogenesis (HA-02-02-01)
ANL-60400, 60402

Use of Liver for Mechanistic Studies of Multistage Hepatocarcinogenesis and for Screening of Environmental Contaminants for Tumor Initiating and Promoting Activity

C. Peraino, K. B. Ekelman, E. Staffeldt, and V. A. Ludeman

The objectives of this project are twofold:

1) Characterization of the stages of tumor formation using liver as the model system.

2) Development of a method for the rapid screening of environmental contaminants for tumor initiating or promoting activity, using the liver as the test system. The realization of this objective requires the identification of reliable indicators of hepatic preneoplasia; the use such indicators is also an essential component of the mechanistic studies that derive from the first objective.

METHODS

Tumorigenesis studies. Rats are fed a diet containing the liver carcinogen 2-acetylaminofluorene (AAF) for a brief interval and are subsequently maintained on a diet containing 0.05% of the hepatic tumor promoter phenobarbital. The rats are then examined first for the appearance of foci of altered hepatocytes, as detected by histochemical and tissue culture techniques, and subsequently for the emergence of detectable tumors.

Screening for environmental initiators and promoters. Male Sprague-Dawley rats weighing 200 grams are partially hepatectomized. Twenty-four hours later the agent to be tested for initiating activity, or a known hepatocarcinogen, is administered by gavage. The rats are then chronically exposed to the known tumor promoter phenobarbital or to the agent to be tested for promoting activity. At intervals, rats are examined for the appearance of foci of altered hepatocytes and, if warranted, for the subsequent appearance of tumors.

Identification and quantitation of altered foci. Serial frozen liver sections are made and assayed histochemically for the appearance of foci containing one of the following alterations: elevated gamma glutamyl-transpeptidase, reduced glucose-6-phosphatase, elevated diaphorase, elevated alkaline phosphatase, reduced iron uptake, increased glycogen storage. By superimposing the images of the successive sections, it is possible to determine the numbers and sizes of foci containing various combinations of the histochemical markers indicated above. This process is facilitated through the use of an image digitizer and computer.

Tissue culture procedures. The tissue culture studies include measurements of biochemical and culture characterisitcs in cells obtained by collagenase perfusion of livers of rats that have been exposed to a variety of carcinogen and promoter combinations.

SUMMARY OF RESULTS

We first demonstrated that the feeding of 0.05% dietary phenobarbital after a brief (2-3 week) feeding of 0.02% dietary AAF enhanced AAF-induced hepatic tumorigenesis several-fold.

Subsequently it was shown that AAF-initiated hepatocytes persist for at least 120 days, and that approximately 250 days of phenobarbital feeding is required for the maximum expression of its promoting effect. DDT and BHT were shown to promote AAF-induced hepatocarcinogenesis in rats and phenobarbital to promote hepatic tumorigenesis in mice genetically susceptible to the spontaneous formation of such tumors.

Our most recent work has shown convincingly that (1) phenobarbital has no intrinsic hepatocarcinogenic activity, and (2) promotion by phenobarbital involves an increase in the probabilty that initiated hepatocytes will express the neoplastic phenotype, with no effect on the character of this phenotype or the kinetics of its expression.

The feasibility of using the liver for analyzing the tumorigenic potential of energy-related pollutants (e.g., polycyclic aromatic hydrocarbons) is being examined. Results to date indicate that the number of altered foci are directly related to the dose of the hydrocarbon benzo(a)pyrene and the duration of the experiment; no foci are observed in partially hepatectomized animals receiving only the phenobarbital diet. An earlier pilot study showed that the dietary phenobarbital treatment following hydrocarbon initiation increased the subsequent incidence of foci fivefold.

CONCLUSION

Our efforts to characterize preneoplastic hepatocytes will investigate several techniques designed to generate tumors differing in their levels of differentiation. These include measurements of (1) tumorigenesis, (2) the occurrence of histochemically altered foci of hepatocytes *in vivo*, and (3) the appearance of properties associated with transformation in hepatocytes cultured from the livers of rats exposed to initiator-promoter treatments. Correlations will be sought among the number and types of tumors that ultimately appear, the spectra of changes occurring in the histochemically altered foci that emerge at successive stages of tumorigenesis, and the proportion of transformed cells in the livers in which the foci are monitored. These correlations should enable us first to identify those carcinogen-altered hepatocytes that are truly preneoplastic, and subsequently to identify the molecular changes within these cells that determine their tumorigenic character.

DOE: Carcinogenesis (HA-02-02-01)
ANL-60400

Molecular Properties of Rat Liver Ornithine Aminotransferase

C. Peraino, W. E. Boernke,* F. Stevens,+ and A. M. Prapuolenis

Considerable effort has been devoted, in this laboratory, to an examination of the unusual adaptive properties of the rat liver mitochondrial enzyme, ornithine aminotransferase (OAT). Part of this work has involved purification and crystallization of OAT as a prerequisite for the preparation of OAT-specific antibody. During the development of our purification procedure, we became aware of the unique molecular properties of this enzyme which enabled its purification by a simple three step procedure. We have, therefore, undertaken an investigation of the physical and chemical characteristics of the OAT molecule, with the expectation that the delineation of these properties will improve our understanding of the mechanism(s) by which OAT is regulated, as well as of the pivotal role this enzyme plays in the control of urea cycle function.

METHODS

Self-association of OAT was studied by measuring the sedimentation characteristics of the enzyme in a Beckman Airfuge under a variety of conditions. Effects of enzyme concentration, presence of substrates and/or inhibitors, and temperature can be measured readily with this technique.

Kinetic experiments involved the determination of the effects of enzyme concentration (degree of self association) on the Km's for ornithine and α-ketoglutarate and on Vmax. In addition, these constants were compared in OAT contained in liver mitochondria prepared from rats fed diets containing 24% or 85% protein.

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SUMMARY OF RESULTS

Our previous studies showed that purified OAT has a strong tendency to aggregate. This property has led to several published inaccuracies in OAT molecular weight assessments. Additional consequences of OAT's aggregation properties are the ease with which the enzyme can be crystallized, and the difficulty encountered in growing a few large crystals suitable for X-ray diffraction analysis, as opposed to many small crystals.

Our current studies have centered on a systematic analysis of the aggregation characteristics of purified OAT as a basis for developing improved crystallization protocols and also to gain insight into the consequences of aggregation for the catalytic function of the enzyme. Analysis of the effects of enzyme concentration on OAT molecular weight, as measured in the Airfuge, indicates that the monomeric form of the enzyme has a molecular weight of 45-50K, in agreement with published data obtained by gel electrophoresis. As the enzyme concentration is raised, the monomers aggregate in a two-stage process involving first the formation of trimers and subsequently the association of trimers to form higher order complexes. The association constant for trimer formation is $K = 3.8 \times 10^{14}$ and that for the higher aggregation states is $K = 6.0 \times 10^{4}$ at pH 8.0, the pH optimum for the enzyme. The tendency to aggregate is reduced at higher pH and increased at lower pH, although the two-stage nature of the aggregation process is not altered by pH changes. Increasing the ionic strength of the enzyme solution by adding KCl also has no effect on the two-stage nature of OAT aggregation, although the degree of aggregation increases as the ionic strength is raised.

Kinetic measurements at different aggregation states of the enzyme showed that increasing the degree of OAT aggregation increased the Km's for ornithine and ketoglutarate but did not change the Vmax, suggesting that OAT aggregation competitively blocks the active site from interaction with the substrates. In rats with elevated OAT levels induced by feeding an 85% protein diet, the Km for α-ketoglutarate, with OAT sequestered in mitochrondria, is significantly higher than that in rats on a 24% protein diet. Sonicating the mitochondria equalizes these Km's. On the basis of the relationship between Km and aggregation state established for the purified enzyme, the mitochondrial Km measurements suggest that induction of the enzyme to high levels *in vivo* is accompanied by an increase in the aggregation state of OAT within the mitochondria.

CONCLUSION

Our observations on the existence of OAT aggregation *in vivo*, and the consequent alterations in the kinetic properties of the enzyme, reveal the existence of a new mechanism for controlling the availability of ornithine for the urea cycle. Thus, OAT aggregation reduces the affinity of the enzyme for ornithine, thereby allowing a greater flux of ornithine through the urea cycle; whereas OAT disaggregation favors its interaction with ornithine, and the consequent diversion of ornithine from its role as a urea cycle intermediate. In addition, our developing understanding of the characteristics of OAT aggregation should eventually enable us to devise optimum conditions for the growth of OAT crystals suitable for X-ray diffraction studies.

DOE: Carcinogenesis (HA-02-02-01)
ANL-60400

Regulation of Gene Expression in Rat Liver

C. Peraino, K. B. Ekelman, and A. M. Prapuolenis

In order to determine the molecular events associated with both the initiation and promotion stages of liver tumor formation, it is necessary to understand the mechanisms by which gene expression is controlled in normal liver. This conclusion arises from the considerable evidence pointing to aberrent gene expression as an invariant biochemical characteristic of neoplasia. Part of our work, therefore, has been directed toward analyzing the control of gene expression in liver, with the expectation that such studies will increase our understanding of the essential molecular differences between normal and neoplastic liver.

Our strategy involves a comparison of changes in the synthesis of the two adaptive enzymes, ornithine aminotransferase (OAT) and serine dehydratase (SDH) in rat liver after exposing the rats to dietary and hormonal stimuli. The results suggest that the range of adaptive responses of these two enzymes is sufficiently broad to enable them to serve as a model for analyzing the control of gene expression in the liver. In using this model two approaches are required. First, in vivo studies are conducted in order to identify the primary regulatory effectors for these enzymes. The second approach involves the development of techniques for analyzing separately the stages of gene expression (i.e., gene transcription, mRNA processing, and mRNA translation) in order to determine the relative degrees of responsiveness of these stages to the actions of the primary effectors. A synthesis of the two approaches should provide information on the nature of the primary effectors and their sites of action. Thus far our efforts have concentrated on the in vivo approach.

METHODS

Rats exposed to adaptive stimuli (hormonal treatments, changes in feeding and lighting schedules) are injected intraperitoneally with ^{3}H-leucine 40 minutes prior to sacrifice. The livers are removed, extracts are prepared, and separate aliquots from each extract are treated with purified antibody prepared against crystallized OAT or SDH. The ratio of antibody precipitable radioactivity to total acid insoluble radioactivity is used as a measure of the relative rate of synthesis for each enzyme.

SUMMARY OF RESULTS

Prior to our synthesis studies, we had observed that glucocorticoid treatment interacted synergistically with increased dietary protein in elevating SDH activity while simultaneously blocking the stimulatory effect of dietary protein on OAT activity. Using antibodies specific to SDH and OAT, we subsequently demonstrated that the glucocorticoid treatment simultaneously induced SDH synthesis and repressed the synthesis of OAT.

During the course of these studies it was observed that the synthesis of both enzymes showed circadian cyclic patterns that appeared to have dissimilar phases. Our most recent studies have compared the circadian synthesis patterns of OAT and SDH in response to changes in feeding and lighting schedules. We observed that, in rats adapted to constant lighting and a single 2-hour feeding interval, the synthesis cycles for both enzymes were clearly evident and were 12 hours out of phase. This relationship persisted irrespective of the clock time of the 2-hour feeding interval.

However, striking changes in the response characteristics of the two enzymes were observed when the 2-hour feeding interval was combined with a 12-hour light-dark cycle. If the feeding interval was located at the beginning of the dark phase, the circadian cycling of SDH synthesis was retained, whereas positioning the feeding interval at the beginning of the light phase produced a low non-oscillating level of synthesis. The behavior of OAT in response to the above protocol was exactly the reverse of that observed for serine dehydratase. Thus, the circadian cycling of OAT synthesis was retained when the feeding interval was located at the beginning of the light phase, but was eliminated when the feeding interval was shifted to the beginning of the dark phase, and the level of synthesis in this non-oscillating state was high. The circadian oscillations for the two enzymes remained 12 hours out of phase under all conditions. The results suggest that biochemical processes that constitute an inductive stimulus for the synthesis of one enzyme serve to repress the synthesis of the other.

In additional studies, we observed that glucocorticoid induced SDH synthesis only when given during the ascending phase of the SDH circadian cycle but repressed OAT synthesis irrespective of the time at which it was given. The latter repressive effect was abolished by inhibitors of transcription (α amanitin and actinomycin D).

CONCLUSION

Our future efforts to analyze the mechanisms underlying SDH and OAT regulation will utilize three approaches.

1) *In vivo* studies: Studies of the effects of light, food, and exogenous hormonal stimuli on OAT and SDH synthesis will continue in an effort to identify the primary regulatory effectors for these enzymes.

2) In vitro studies: The in vivo studies will be accompanied by in vitro experiments utilizing rat hepatocytes maintained in primary culture and a neoplastic hepatoma cell line. A comparison of the responses of OAT and SDH in vivo and in vitro will be undertaken as a means of assessing which aspects of the regulation of these enzymes are purely cellular and which require the integrity of the organism.

3) Assays for mRNA: Using a cell-free protein synthesizing system (e.g., wheat germ or reticulocyte lysate), assays for OAT and SDH mRNA levels will be devised. It will then be possible to determine whether a given adaptive response (change in OAT and/or SDH synthesis) results from the modulation of transcriptional or posttranscriptional processes.

DOE: Carcinogenesis (HA-02-02-01)
ANL-60401

Methods of Tumor Detection

T. E. O'Connor, B. A. Sedita, G. G. Martin,* S. P. Binette,† S. R. Gawne‡ S. M. Holland,** D. Miller,†† and S. P. Singh‡‡

The objective of this project is the development of markers of neoplasia to detect cancer at earlier stages than heretofore possible. The markers should prove useful in studying the etiology and progression of experimental and spontaneous tumors and, ultimately, in early detection of neoplasia in human populations.

METHODS

Our experimental system uses carcinogen-induced, primary rat hepatocarcinomas, transplantable Novikoff rat hepatoma, and normal rat liver. Biochemical and immunological characteristics of chromatins of normal rat livers and hepatocarcinomas are examined, and procedures are developed for detection and quantitation of cancer-specific alterations. Xenogeneic antisera are raised in rabbits to normal rat liver and tumor chromatin fractions. These antisera are employed in characterization of the chromatin antigens in procedures involving Levine's micro complement-fixation, indirect fluorescent antibody techniques, and Grabar-Williams and "ISOL" immunoelectrophoresis. Proteins are characterized by PAGE-SDS electrophoresis, "ISO-DALT" two-dimensional electrophoresis, and isoelectric focusing procedures (including the newly developed ISOL procedure).

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SUMMARY OF RESULTS

Complement fixation studies indicate that chromatins from Novikoff hepatoma and primary hepatocellular carcinomas induced by 2-acetylaminofluorene (AAF) contain equivalent amounts of antigens, not detected in chromatins of normal adult rat livers.

The fluorescent antibody procedure specifically stains nuclei of either Novikoff hepatoma or AAF-induced hepatocellular carcinoma cells, but not nuclei of normal rat livers.

The sequential extraction of proteins from chromatins, coupled with complement fixation and PAGE-SDS analysis of the chromatin residual fractions, indicates that the tumor-specific antigenic determinants are associated with a small number of proteins that tightly bind to DNA.

Two-dimensional ("Grabar-Williams") immunoelectrophoresis of Novikoff hepatoma nonhistone chromosomal proteins (NHCP's) with the anti-Novikoff serum gave a simple pattern of specific precipitin bands, but interpretation of this finding is constrained by technical difficulties associated with NHCP's.

A two-dimensional procedure ("ISOL") that separates proteins in aqueous-agarose media by isoelectric focusing followed by immunoelectrophoresis has been developed. The ISOL procedure readily characterizes serum proteins without denaturation. Problems associated with ISOL characterization of tumor-specific chromatin antigens, due to solubility characteristics of NHCP's, are under study.

CONCLUSION

Neo-chromatin antigens apparently specific for hepatic tumors have been detected.

DOE: Carcinogenesis (HA-02-02-01)
ANL-60500

National Cancer Institute Contract
Y01 CP 7-0504
ANL-8D405

Mechanisms of Radiation and Viral Oncogenesis

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This project is concerned with the health hazards of energy-related environmental pollutants, focusing on molecular mechanisms of oncogenesis. Present objectives are to elucidate the role of endogenous retroviruses and the overall mechanism in radiation-induced murine osteosarcoma model systems. Through animal model studies, we aim to provide insight on how silent malignant host genes can be turned on as a result of exposure to various biological, physical, and chemical agents, individually and in combination.

METHODS

In these model systems, osteosarcomas can be induced independently in CF#1 and X/Gf mice, by either a bone-seeking radionuclide, ^{90}Sr, or a viral agent, FBJ in CF#1 mice, and FBR in X/Gf mice. To show a viral involvement in the genesis of ^{90}Sr osteosarcoma, two approaches were undertaken. The first seeks to confirm the presence of viruses in ^{90}Sr-induced osteosarcomas, and the second applies molecular techniques to

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demonstrate the activation of an endogenous viral and malignant gene (sarcogene). These comprehensive approaches examine events ranging from synthesis of gene protein products to development of tumors in animals, and involve various techniques of immunology, biochemistry, virology, tissue culture, and animal experimentation.

SUMMARY OF RESULTS

Virus in ^{90}Sr-Induced Osteosarcomas

A number of viruses have been isolated from ^{90}Sr osteosarcomas, 36 from CF#1 and 21 from X/Gf mice. These new isolates are polymerase-positive, infectious, XC-plaque-inducing but nontransforming *in vitro*. *In vivo*, however, they do induce some osteosarcomas, although the latent period is much longer and the incidence is lower than in the case of FBJ and FBR viruses.

The associated nontransforming FBJ and FRB virus stocks, propagated in the absence of the transforming components, have *in vitro* and *in vivo* properties similar to the new virus isolates. These data are consistent with a working hypothesis that endogenous nontransforming viruses are first activated by ^{90}Sr, then, through a recombination event, the replicating viral genome excerpts a silent transforming gene (sarcogene) of the host, thereby effecting the continued expression of the sarcogene and leading to osteosarcomas.

Molecular Studies

The FBJ and FBR viruses have been partially characterized. They possess biophysical, biochemical, and immunologic properties characteristic of mammalian type C retroviruses. Both infect only murine cells and do not infect cells from a wide range of nonmurine species. FBJ virus is NB-tropic, whereas FBR virus is distinctly B-tropic.

No xenotropic viruses were detectable in association with either virus stock.

Although yielding single-hit focus induction kinetics, these sarcoma viruses are replication defective, because transformed nonvirus producer cell clones could be isolated.

Genomically and antigenically, FBJ and FBR viruses are very closely related to the endogenous ecotropic viruses of AKR and BALB/c mice, but are completely unrelated to six nonmurine retroviruses tested.

DNA probing studies of host genomes indicate both FBJ and FBR are in fact endogenous viruses.

In the course of these studies we discovered the enhancing effects of polyethylene glycol on reverse transcription and described conditions for increasing the yield of ^{3}H-cDNA probes in such synthetic reactions.

CONCLUSION

To confirm our working hypothesis, we aim to develop specific DNA probes and immunologic assays for the *in vivo* monitoring of virus activation and sarcogene expression following administration of ^{90}Sr. In addition, attempts will be made to identify and characterize the sarcogene sequences and to reproduce the recombination event *in vivo* and *in vitro*.

DOE: Toxciology of Coal
Technologies (HA-02-02-03)
ANL-60405

Biphenyl Metabolism by Rat Liver Microsomes: Regioselective Effects of Inducers, Solvents, and Inhibitors

D. A. Haugen* and K. Suhrbier*

A detailed investigation of the metabolism of biphenyl was undertaken in order (1) to understand better the cytochrome P-450-dependent monooxygenases responsible for its metabolism, and (2) to evaluate the use of biphenyl metabolism both as a tool in the characterization of microsomal and purified monooxygenases, and in the examination of the effects of putative environmental agents on the monooxygenase system. The monooxygenases investigated in this study are responsible for the metabolic activation of a wide variety of carcinogenic and otherwise toxic chemicals to biologically active electrophilic metabolites ultimately responsible for the adverse effects of the agents. The metabolism of biphenyl is typical of that for many aromatic hydrocarbons, including those derived from fossil fuel combustion and conversion technologies.

METHODS

Initial oxidation of biphenyl yields each of the three possible monohydroxy derivatives, 2-, 3-, and 4-hydroxybiphenyl. Modification of the reaction conditions, and pretreatment of animals with various agents may alter the distribution of metabolism at the three regions of the molecule, i.e., the regioselectivity is altered. In the present study, we examined the effects on the regioselectivity of biphenyl metabolism of (1) pretreating rats with the inducers phenobarbital or 3-methylcholanthrene, and (2) the presence in microsomal reaction mixtures of the solvents methanol, acetone, or dimethyl sulfoxide, or (3) the presence of the inhibitors 7,8-benzoflavone or 1-benzylimidazole.

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*Toxicology Program for Coal Combustion and Conversion Effluents.

SUMMARY OF RESULTS

Phenobarbital pretreatment primarily induced 2- and 3-hydroxylation, the latter most dramatically (up to 30-fold increase over nontreated animals under certain conditions). 3-Methylcholanthrene pretreatment induced 2- and 3-hydroxylation to similar extents (10- to 15-fold increase over nontreated animals). The inhibitors and solvents had regioselective effects on biphenyl metabolism that are characteristic of the uninduced, phenobarbital-induced, and 3-methylcholanthrene-induced microsomes. The presence of multiple forms of cytochrome P-450 in uninduced microsomes is indicated by the regioselective effects of the solvents and the inhibitors. The 3-methylcholanthrene-dependent increases in 2- and 3-hydroxylation appear due to induction of a single form of cytochrome P-450 as indicated by similar dose-response relationships and similar changes in sensitivity to the inhibitors. The phenobarbital-dependent increases in 2- and 3-hydroxylation appear due to the induction of two forms of cytochrome P-450 as indicated by different dose-response relationships and by different changes in sensitivity to the effects of dimethyl sulfoxide and 7,8-benzoflavone.

The results indicate that examination of the regioselectivity of biphenyl metabolism is a useful approach for characterizing microsomal monooxygenases, and they suggest that the approach may also be useful in the characterization of purified monooxygenase systems. Furthermore, the dose-response relationships for the induction of biphenyl metabolism demonstrated that increases in biphenyl metabolism can be used as a sensitive measure of exposure to agents that induce the monooxygenase system. Such an approach may be superior to the more commonly employed measurements of benzo(a)pyrene metabolism using a fluorometric assay.

CONCLUSION

The regioselective effects of inducers, solvents, and inhibitors on biphenyl metabolism by rat liver microsomes were studied. The data demonstrated that use of measurement of biphenyl hydroxylation is potentially a useful tool in characterizing purified monooxygenases and in assessing the effects of environmental agents on the monooxygenase system.

DOE: Toxicology of Coal
Technologies (HA-02-02-03)
ANL-60405

Aryl Hydrocarbon Hydroxylase: Degradation of Phenolic Metabolites Due to Contaminant in Acetone

D. A. Haugen* and K. Suhrbier*

In this laboratory, measurement of benzo(a)pyrene metabolism by application of the widely used fluorometric aryl hydrocarbon hydroxylase (AHH) assay revealed that the metabolites were unstable under the final conditions of the assay only if they had not previously been subjected to the preliminary procedures in the assay. This study was undertaken to explain this observation.

METHODS

The AHH assay is used frequently in the areas of pharmacology, drug metabolism, cancer research, and environmental toxicology. In this assay, the enzymatic reactions are stopped by addition of acetone, and the metabolites are extracted first into hexane and then into 1 N NaOH. Fluorescence of the phenolic metabolites [principally 3-hydroxybenzo(a)pyrene] is then measured in the sodium hydroxide solution.

SUMMARY OF RESULTS

When 3-hydroxybenzo(a)pyrene was placed directly in 1 N NaOH, its fluorescence decreased only 5 to 10% per hour. However, when it was placed into blank reaction mixtures, carried through the assay procedure, and finally extracted into 1 N NaOH, its fluorescence decreased up to 70% in an hour.

Systematic elimination of the various steps and reagents employed in the assay revealed that a contaminant was present in reagent grade acetone, from several sources, that was solely responsible for the degradation of

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*Toxicology Program for Coal Combustion and Conversion Effluents.

both the standard 3-hydroxybenzo(a)pyrene and the enzyme-dependent products. The acetone could be readily purified by distillation. Although the contaminant(s) was not identified, its properties suggest that it is a relatively volatile organic material, possibly an oxidizing agent.

CONCLUSION

An unidentified contaminant present in reagent grade acetone causes degradation of fluorescent phenolic metabolites of benzo(a)pyrene as measured in the widely used aryl hydrocarbon hydroxylase assay. The acetone may be readily purified by distillation. Use of purified acetone to stop the enzymatic reactions will allow this assay to be performed with greater ease and reliability.

National Institute of Arthritis, Metabolism and Digestive Diseases Grant AM 21592
ANL-85809

National Cancer Institute Grant CA 21556
ANL-85866

13. Liposomes as Biological Carriers: New Therapeutic Approaches to Metal Toxicity and Malignant Tumors

Y. E. Rahman,* E. H. Lau,* K. R. Patel,* E. A. Cerny,* and B. J. Wright*

This program aims to develop a new technique of drug encapsulation within liposomes to deliver metal chelating agents and antitumor drugs to specified target organs in order to:

1) enhance the therapeutic effect of the drug,

2) reduce the drug toxicity to nontarget normal organs, and

3) reduce the effective dosage of the drug.

METHODS

We use a carrier made of natural membrane lipids, namely a microscopic capsule called a "liposome," to encapsulate drugs either in the aqueous compartments or in the lipid bilayers. To achieve liposome targeting, multilamellar and unilamellar liposomes of different sizes and different lipid composition, particularly glycolipids, are prepared. Our experiments are based upon the recent findings from several laboratories that specific carbohydrate-mediated recognition sites are found on the surface of various cell systems. Cell types in various organs that are responsible for high affinity to a specific type of liposomes are identified by use of labeled drugs, cell fractionation, and autoradiographic techniques. Selected metal chelating agents or antitumor drugs encapsulated within a specific type of liposome are used for therapeutic experiments, and their efficacy in mice is compared with that of the corresponding free, nonencapsulated drug.

*Carcinogenesis Group.

SUMMARY OF RESULTS

The chelating agent DTPA (diethylenetriaminepentaacetic acid) encapsulated in liposomes was shown to be more effective than the nonencapsulated drug in removing plutonium, lead, and mercury from mouse liver, bone, and kidney.

Smaller sized, unilamellar liposomes were more effective than larger sized, multilamellar liposomes for removing storage iron from the parenchymal cell compartment of the liver, whereas multilamellar liposomes were more effective in removing iron from the Kupffer cell compartment.

Liposomes made with a glycolipid containing a galactose moiety, e.g., galactocerebroside, were more effective than liposomes without the glycolipid in removing storage iron from the parenchymal cells of the liver, whereas liposomes without the glycolipid were more effective for removing iron from the Kupffer cell compartment.

Liposome-encapsulated actinomycin D, cytosine arabinoside, and Methotrexate were shown to be significantly more effective than the nonencapsulated drug in improving the survival time of mice bearing Ehrlich ascites tumor, Lewis lung carcinoma, and hepatoma 129 cells.

CONCLUSION

We have demonstrated that by manipulating the size and lipid composition of liposomes, selective delivery of liposome-encapsulated metal chelators to either the parenchymal or the Kupffer cells of the liver can be achieved. Based on the recent progress made in the field of cell surface receptors, liposomes containing known receptor groups will be targeted to specific cell compartments of liver, and other organs such as spleen and bone marrow.

REGULAR STAFF

Elizabeth A. Cerny (Scientific Assistant)
Yueh-Erh Rahman (Biologist)
Betty J. Wright (Scientific Associate)

TEMPORARY STAFF DURING 1979

Ellen H. Lau (Research Associate)
Kanaiyalal R. Patel (Postdoctoral Appointee)

14. Molecular Anatomy Program: Molecular Perturbations in Man Produced by Energy-Related Pollutants

N. G. Anderson, N. L. Anderson, J. J. Edwards, C. S. Giometti, B. S. Coulter,* B. J. Hickman, L. J. Ross,† A. Scandora,‡ J. Taylor,* and K. E. Willard**

The goals of this program are as follows:

1) Analysis by high-resolution two-dimensional electrophoresis of human cells, tissues, and body fluids to detect alterations caused by toxic chemicals, including carcinogens, tumor promoters, mutagens, teratogens, and heavy metals, and by radiation.

2) Preparation of a protein index for man.

3) Development of computerized data reduction systems for two-dimensional electrophoresis data.

4) Development of internal standards to define spot positions and of systems for analyzing large numbers of samples and for recording data.

METHODS

The ISO-DALT systems for doing multiple parallel two-dimensional electrophoretic analyses (Anderson, N. L., and N. G. Anderson, Proc. Nat. Acad. Sci. 74, 5421, 1977; Anderson, N. G., and N. L. Anderson, Anal. Biochem. 85, 331, 1978; Anderson, N. L., and N. G. Anderson, Anal. Biochem. 85, 341, 1978) have been developed to the point where as many as 100 analyses can be

*Applied Mathematics Division.

†Participant in the 1979 Summer Institute in Biology, Rochester Institute of Technology.

‡Science Applications, Inc.

**Resident Student Associate, Virginia Polytechnic Institute and State University.

done per day. New methods for obtaining analytical data on gel spots, and for identifying activities have been developed. Methods and procedures for indexing protein gene products have been worked out that list positional coordinates with reference to standards and available analytical data. Each spot has a serial number, as well as one or more common names, and represents a unique protein having a variety of determinable properties.

Methods have been devised for characterizing cellular proteins en masse, in the sense that some characteristic can be determined for all the spots on the gel simultaneously. Three types of characteristics can currently be obtained: (1) cellular location (surface, cytoskeletal, mitochondrial, nuclear, or soluble); (2) physicochemical properties (approximate amino acid composition, phosphate content, thermostability, SH content, interaction with small molecules); and (3) regulatory behavior (regulation by effectors, alteration during differentiation, etc.).

Computerized data reduction systems now enable rapid noise subtraction, background flattening, resolution of spots into two-dimensional Gaussian curves, normalization of a spot pattern with reference to a standard, and the preparation of spot lists. Software has been developed capable of fitting five parameter two-dimensional Gaussians to 1200 spots simultaneously. Gaussian fitting is a good method of modeling 2-D gel spots since diffusion in each of the dimensions produces a final spot shape very close to the product of two orthogonal Gaussian forms.

SUMMARY OF RESULTS

We have built the facilities and developed the techniques to index a large fraction of human protein gene products (PGP's).

A central question is whether order can be made of the numerous alterations to be seen, i.e., whether PGP's are regulated in discernable sets, and whether the regulational mechanisms can be discovered. Our major finding is that specific groups of PGP's are affected by many specific agents.

Using human peripheral blood lymphocytes, where 500-1000 PGP's may be routinely detected, the following substances have been shown to affect the number of PGP's shown in parentheses: poly I:C or interferon (5-8), phorbol ester tumor promoter (20), intracellular Ca++ using the ionophore A23187 (10), dexamethasone (1), $CdCl_2$ (2-12), colchicine (2), ouabain, (3-12), and mitochondrial poisons, including valinomycin and oligomycin (7).

We have discovered that the synthesis of mitochondrial proteins (both those encoded by the nucleus and the mitochondria) is controlled by mitochondrial function (inhibited by antimitochondrial agents). In the case of the nuclear-encoded genes, control is apparently by means of a translational mechanism. At least two major differences exist between normal peripheral blood lymphocytes and transformed lymphoid cell lines as regards synthesis of mitochondrial proteins.

Phorbol myristate acetate (PMA) induces a strict subset of the changes associated with transformation, the phosphorylation of nonmuscle myosin light chains, and the synthesis of one mitochondrially encoded protein. Raised intracellular calcium induces about half the PMA subset.

These results show how tumor promotion, carcinogenesis, and the effects of a wide variety of toxic agents, and of radiation, may now be redescribed and understood at the level of the control of the expression of specific describable genes.

Approximately 40 investigators have been trained in this laboratory in 2-D gel techniques. Two companies now produce elements of the original ISO-DALT system commercially and clinical implementation of such systems is proceeding rapidly, particularly in Europe.

CONCLUSION

The Molecular Anatomy Program's ISO-DALT facility remains the largest two-dimensional gel system in existence (100 gels/day, > 25,000 run to date). Continuing efforts are being made in the areas of (1) resolution of basic proteins (BASO-DALT procedure), (2) standardization with charge and molecular weight standards, (3) improved autoradiographic and fluorographic techniques, (4) exploitation of solid-phase transfer methods (DBM-paper, etc.) for the preparation of antigenically reactive 2-D replicas, and (5) exploration of thin-film techniques.

The detection of new mutations, of existing disease-associated variants, and of molecular alterations induced by energy-related toxic substances can now be done on very small samples obtained from human populations.

REGULAR STAFF

Norman G. Anderson (Senior Physiologist)
N. Leigh Anderson (Assistant Biophysicist)
Barbara J. Hickman (Scientific Assistant)
Sharron L. Nance (Scientific Associate)
Sandra L. Tollaksen (Scientific Associate)

TEMPORARY STAFF DURING 1979

Jesse J. Edwards (Postdoctoral Appointee)
Carol S. Giometti (Postdoctoral Appointee)

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ANL-8D403

Clinical Research Resource, NIH Interagency Agreement
ANL-8D406

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ANL-85665

15. Argonne Bioanalytical Center: Development of New Technology for the Use of Stable Isotopic Tracers in the Study of Human Health and Disease

P. D. Klein, P. A. Szczepanik-Van Leeuwen, D. L. Hachey, B. R. DeMark, C. S. Irving, H. C. Niu, and F. Stellaard

The Argonne Bioanalytical Center was developed over the last ten years to promote the use of stable isotopic tracers in biomedical and clinical research. It seeks to replace the use of ^{3}H and ^{14}C with the nonradioactive isotopes ^{2}H, ^{13}C, ^{15}N, and ^{18}O, in order to enable diagnostic and research studies in populations of infants, children, and pregnant women, who are excluded from investigations using radioisotopes.

This program has five major aspects:

1) The development of analytical instrumentation to conduct stable isotope measurements in a routine manner.

2) The development of appropriately labeled compounds for metabolic investigations.

3) Development of analytical methodology to isolate, purify, and determine the isotopic content of specific organic compounds reflecting metabolic or disease states.

4) Collaborative development of clinical applications and testing on a routine basis, through a network of clinical centers.

5) The collection and dissemination of stable isotope information on an international scale through survey publications and conferences.

INTRODUCTION

A number of separate programs have comprised the Stable Isotope Program: the Argonne Bioanalytical Center, in the Division of Biological and Medical Research, ANL; the NIH grant to study Bile Acid Transformations in Gallstone Therapy (to 12/31/77); the National Cooperative Gallstone Study ancillary studies; the NIH-GCRC contract for gas chromatography-mass spectrometry assistance to clinicians; and the NIH grant to the Department of Medicine, University of Chicago, for ^{13}C applications to digestive diseases. During 1979 the decision was made to move the Argonne Bioanalytical Center to the Children's Nutrition Research Center at Baylor College of Medicine, in Houston, Texas. Our efforts have therefore been directed to completing the responsibilities associated with the currently funded programs, and with preparing to transfer the NIH grant from the University of Chicago to Baylor College of Medicine. It is expected that the Argonne Bioanalytical Center will complete its move during 1980.

The following review serves as a final report for the Stable Isotope Program. It summarizes the many and varied accomplishments of the program, with the emphasis on the last 3 years. Our earlier work developed the instrumentation and methodology and synthesized many of the compounds labeled with stable isotopes.

A special acknowledgment should be made of the many significant contributions of the Postdoctoral Appointees who have worked with the group since 1974. Bruce R. DeMark, Charles S. Irving, Hsien-Chi Niu, and Frans Stellaard, whose period of tenure included 1979, are coauthors of this report. William Bryant, Karin Mede, Dale A. Schoeller, and Kou-Yi Tserng served in previous years. In addition, we acknowledge the substantial contributions of the foreign Visiting Scientists who have worked in the program.

METHODS

Three gas chromatographs/mass spectrometers (GC/MS), all equipped with selected ion ratiometers and interfaced to a PDP-12 computer system, provide the basis for the methodology. In addition, two gas isotope ratio analyzers, one equipped with an automated CO_2 purification system developed at Argonne, have been used for the analysis of breath CO_2.

SUMMARY OF RESULTS

Bile Acid Studies

The Bile Acid grants have enabled us to develop new methods of bile acid identification and quantitation, using gas chromatography-mass spectrometry, as well as to prepare a series of bile acids labeled with the stable isotopes deuterium and carbon-13.

A unit for stable isotope ratio measurements and multiple ion monitoring, used for stable isotope kinetic studies of bile acid metabolism has been designed, constructed, and used on a daily basis.

A new separation of bile acids as their phenacyl esters has been developed using high pressure liquid chromatography.

The electron impact and chemical ionization mass spectra of more than 50 bile acids and their derivatives have been acquired and analyzed for characterization of complex mixtures.

The gas chromatographic retention time of these bile acids on several liquid phases has been mapped, to provide a retention index coordinate in their identification.

New procedures for the preparation of 24-^{13}C-labeled bile acids have been developed, as well as for the preparation of 3-keto bile acids and formyl esters of bile acids.

A series of sulfated bile acids as the free acid, glycine and taurine conjugates has been prepared for the first time and the physical characterisitcs have been determined.

The lability of keto bile acids during isolation has been documented and new techniques for preservation of the original content have been developed.

The bile acid composition of patients undergoing chenodeoxycholic acid therapy for gallstone dissolution has been examined and shown to contain no unusual or novel forms as a consequence of treatment.

A number of diverse studies have dealt with the measurement of bile acid kinetics in health and disease:

Several studies have aimed at establishing the factors that increase the risk of gallstone formation in women. Studies of bile acid kinetics in pregnant women during all three trimesters of pregnancy and postpartum indicated that bile acid composition is drastically altered during pregnancy. In women on contraceptive steroids, a similar change in composition, as well as an increase in cholesterol secretion occurred, probably major contributing factors to gallstone formation in women taking these steroids.

Pool size determinations of chenodeoxycholic acid have shown that there is a difference in bile lithogenicity in Pima Indian children during their prepubertal, pubertal, and postpubertal stages of development. These data may help to elucidate the cause of the high incidence of gallstones in American Indians.

Because taurine is essential for infant development, we studied the effect of breast milk vs. synthetic diets on the growth and development of newborn infants and whether taurine and/or taurine plus cholesterol affect the synthesis and interluminal concentration of bile acids. The pool sizes and fractional turnover rates of chenodeoxycholic and cholic acids were determined. The most significant conclusion was that bile acid pool sizes were greater in infants fed breast milk. Similar increases could not be achieved with taurine or taurine plus cholesterol supplementation. Bile acid synthesis rates, however, were constant despite changes in diet, indicating that they were maximal in all groups.

Studies are under way to determine the effect of phenobarbital and cholestyramine therapy on hepatic excretory function and bile acid kinetics in children with intrahepatic cholestasis.

The bile acid program has included studies to determine the specific defect in several genetic (and usually fatal) cholestatic liver diseases in children:

Analysis of the biological fluids from children with Zellweger's syndrome uncovered evidence of several C-27 bile acids--trihydroxycoprostanic, dihydroxycoprostanic, and varanic acids. The presence of these compounds suggests that mitochondrial oxidation is necessary for the formation of primary C-24 bile acids and indicates that a defect in the mitochondrial function may contribute to this disease.

Bile, serum, and urine samples from children with Byler's disease, a genetic cholestatic liver disease, were examined. Hyocholic acid was detected in the fluids of all patients examined; it was not present in samples from 20 infants with nonfamilial cholestatic liver disease. In addition, hyocholic acid, β-muricholic acid, and four compounds thought to be tetrahydroxy-bile acids were identified in the bile, serum, and urine of one child from samples taken over a period of several years.

We have recently also detected hyocholic acid in the serum of patients with biliary atresia. This compound may be a marker for differentiating cholestatic disorders with a potential for cirrhosis from neonatal hepatitis.

Because serum concentration of hyocholic acid is thought to be increased in cholestatic patients after phenobarbital treatment, we analyzed serum bile acids from patients with primary biliary cirrhosis who showed differences in reduction of their serum bile acid levels in response to phenobarbital treatment. A failure to detect hyocholic acid in the serum of these patients suggests that 6-hydroxylation is not responsible for the changes in bile acid concentrations observed with treatment.

Methadone Metabolism

Metabolism of methadone in patients being treated for heroin addiction by methadone maintenance therapy is particularly suitable for study by stable isotope techniques:

Studies of d_5 methadone in patients showed the methadone pool has two components. A small component is cleared from the body with a half-life of 5-7 hours, but the majority is cleared very slowly with a half-life of 30-50 hours.

Methadone is used clinically as the racemic mixture of two optically active forms. Pharmacokinetic measurements within the same patient have shown that the inactive (+) methadone is eliminated from the body almost twice as readily as the active (-) form, and the removal of the racemate closely approximated that of the average of the individual isomers.

Plasma drug level pharmacokinetic studies using multiple simultaneous stable isotopic tracers yielded data that are consistent with our earlier findings that the two enantiomers of methadone are metabolized differently. Several studies have been completed in methadone maintenance patients who have liver disease and have a history of alcohol abuse.

Metal Stable Isotopes

Metal stable isotopes are powerful tools for studying metabolism of trace metals that are essential for human nutrition or that are toxic by-products from energy production.

Techniques have been developed to ash biological samples using strong oxidizing acids and to analyze the metals as volatile chelates. Using Schiff bases and other chelating agents, metal chelates have been prepared for magnesium, calcium, iron, copper, zinc, and cadmium to test their suitability for mass spectral isotope ratio measurements. These studies of magnesium and calcium indicate that it is possible to obtain precise isotope ratio measurements (± 0.06%) and accurate natural abundance measurements (± 0.2-0.8%) in plasma and urine.

For analysis of transition metals (iron, copper, zinc), a much wider selection of chelating agents and ionization techniques is available. Isotope ratio studies have achieved a precision of ± 0.05-0.30% for simultaneous measurement of all five naturally occurring zinc isotopes. Similarly, we have obtained experimentally precise simultaneous measurement of all eight cadmium isotopes by chemical ionization-mass spectrometry.

Breath Tests

The development of clinically useful ^{13}C breath tests has continued:

Evaluation of three $^{13}CO_2$ breath tests employing ^{13}C-labeled fats (^{13}C-labeled triolein, trioctanoin, and palmitic acid) in normal children and in children with disorders has been completed. The triolein breath test was demonstrated to be a superior screening test for fat malabsorption. The palmitic acid breath test, in combination with the triolein breath test, was specific for pancreatic disorders and may be a noninvasive alternative to the pancreatic stimulation test which requires intubation. The trioctanoin breath test was the least specific, giving poor separation of children with mucosal or intraluminal disorders from normals.

Three new CO_2 breath test substrates have been investigated. O-alkyl-labeled methacetin and phenacetin have been compared with aminopyrine for the measurement of liver function. The three breath tests all gave good correlations with the degree of liver disease; however, the phenacetin was too slowly absorbed to be practical, and methacetin metabolism was significantly induced by common stimulants such as cigarette smoke and caffeine, causing some overlap in response for normals and abnormals. Aminopyrine remains the preferred substrate for testing liver function. Methacetin may prove to be useful in studies of liver induction. The third substrate, ^{13}C-lactose, has been validated against its ^{14}C analogue; the ^{13}C and ^{14}C results were equivalent.

The aminopyrine breath test for the detection of liver disease in subjects on methadone maintenance and for the prediction of maintenance dose requirements showed good correlation with liver disease. Thus, methadone does not greatly alter aminopyrine metabolism.

The effects of increased fiber intake on the absorption and utilization of glucose were studied. Although previous studies have shown that the addition of fiber to a meal has a significant effect on plasma glucose and plasma insulin levels, no significant change in either the rate or amount of glucose utilization could be detected. Thus fiber does not seem to cause a change in the ultimate utilization of the glucose or in the amount of glucose not absorbed from the intestine.

A fecal assay for the detection of unabsorbed bile acids has been developed and applied to the detection of bile acid malabsorption in children. The test can detect less than 1% bile acid malabsorption. The fecal test is used in conjunction with a breath $^{13}CO_2$ test to detect intestinal bile acid deconjugation in cases where neither test alone establishes diagnosis.

A study of the utilization of intravenously administered lipid using naturally labeled lipids has shown that only about one half of the lipid utilized during the 3-hour lipid infusion and the following 3-hour period was from the infused lipid. The remainder was endogenous lipid, presumably from fat stores.

A 2-year longitudinal study of liver function in patients after jejunal/ileal bypass surgery for the treatment of morbid obesity was begun to compare the aminopyrine breath test with standard liver function tests. The presurgery aminopyrine breath test of a subject who developed hepatic complications was lower than the 14 who did not, and breath tests showed a further loss of liver function before the other liver tests indicated hepatic complication.

Assessment of carbohydrate malabsorption from the intestine, a disorder that may have serious consequences on the patient's nutritional status, is now possible through very precise measurements of breath hydrogen. We have refined previous gas chromatographic procedures for separation of hydrogen from other breath constituents, and improved its quantitation to the point where hydrogen can be detected in concentrations as low as 2 parts per million in expired air. It can be quantitated at levels of 5 ppm, and at levels of 120 ppm the precision is 1%.

Recent reports indicated that ^{18}O-labeled water could be used to provide a noninvasive technique for estimating total body water. This relies on the rapid equilibrium established between the oxygen isotopes of water and those of CO_2. We have compared ^{18}O measurements with $^{2}H_2O$ measurements in the same individual, in preparation for clinical studies in patients with morbid obesity undergoing jejunal-ileal bypass operations and in women during pregnancy.

Other Studies

Taurine (aminoethane sulfonic acid) is a product of cysteine metabolism which has been postulated to be an essential amino acid for the neonate human. It is an ideal compound for amino acid kinetic measurements because it is not further metabolized. Methods were developed for quantitative derivatization and for GC-MS analysis of taurine, and isotope ratio measurements were carried out on standard samples. These show that it should be possible to detect taurine at dilutions as low as 1 part in 1000 to 1 part in 3000 in samples of 10 micrograms.

The response of patients with epileptic seizures to drug treatment is difficult to predict or assess on the basis of dosage. Plasma levels of the parent drug may be of less consequence than drug levels of a particular metabolite. Derivatization, GC separation, and mass spectrometric identification of phenobarbital, phenytoin, valproic acid, and several other antiepileptic compounds have been carried out in order to assess the response of epileptic seizures to drug treatment. Detection by selected ion monitoring and quantitation by GC-MS-computer techniques have been done on standard mixtures.

Information Dissemination

Three International Conferences on Stable Isotopes were organized by this group, and the proceedings have been published. The last was held May 23-26, 1978 at Oak Brook, Illinois. Papers dealt with environmental, clinical, pharmacological, biochemical, and methodological applications of stable isotopes.

An update of the selected bibliographies of biomedical and environmental applications of ^{2}H, ^{13}C, ^{15}N, ^{17}O, ^{18}O, and ^{34}S was completed. Every 2 years there is an increase of 50% in the numbers of publications dealing with stable isotopes; this number may be even greater for papers dealing with multiple isotope usage in environmental applications.

Training and experience in the application of stable isotopes and GC-MS to clinical and biochemical problems has been given to a number of foreign Visiting Scientists under the sponsorship of their home institutions.

REGULAR STAFF

David L. Hachey (Chemist)
Peter D. Klein, (Senior Biochemist)
Patricia A. Szczepanik-Van Leeuwen (Biochemist)

TEMPORARY STAFF DURING 1979

Bruce R. DeMark (Postdoctoral Appointee)
Charles S. Irving (Postdoctoral Appointee)
Hsien-Chi Niu (Postdoctoral Appointee)
Frans Stellaard (Research Associate)

16. Support Facilities

ANIMAL FACILITIES

J. G. Linsley, T. E. Fritz, P. C. Brennan, W. G. Keenan, C. M. Poole, R. C. Simkins, and D. Tolle

The Laboratory Animal Facilities comprise approximately 33.8% (39,532 square feet) of the total usable space within the Division. The major facility is located in Building 202 and a smaller facility, limited to viral oncology studies, is in Building 340.

The closed breeding colony of beagles occupies 9,630 square feet. Six kennels contain 380 runs and housed an average of 755 dogs in 1979. Each run consists of a heated indoor compartment plus an outdoor exercise area. Technical equipment areas allocated for the support of the dog programs occupies another 8,230 square feet. These include three gamma exposure rooms, two dog treatment rooms, an operating room, necropsy room, and X-ray room. The remaining 45% (17,860 square feet) of the Animal Facilities consists of 47 animal rooms, and rooms for cage and bottle washing, sterilization, and injection of radioactive materials. Each of the animal rooms is capable of holding laboratory animals under controlled conditions of temperature, humidity, and lighting.

Renovations and modifications of the dog kennels were begun in the fall of 1979. The renovations included removing the fiber glass tub liners and installing new run dividers, gutters, doors, and gates. The design of the new dividers and gates will enable the use of automated cleaning equipment and will result in more efficient cleaning procedures and better sanitation.

The 26 animal holding and breeding rooms located in "E" wing which were renovated over the past 2 years were reoccupied in 1979.

The installation of two new sterilizers (one steam and one gas) and the modification of an existing steam sterilizer, all located in "E" wing, should be completed by July of 1980.

During 1979, approximately 22 staff members of the Division were involved in various studies utilizing in excess of 30,000 rodents and other laboratory animal species.

The supporting facilities for the husbandry and clinical monitoring of the resident animals and the responsible personnel include:

1) Automated cage and bottle washing and filling machinery (Animal Care Specialists)

2) Steam and gas autoclaves for sterilization of equipment, instruments, and media

3) Diagnostic X-ray facilities and darkrooms (C. M. Poole, W. G. Keenan)

4) Clinical pathology laboratory (D. V. Tolle, R. C. Simkins)

5) Hematology laboratory (D. V. Tolle)

6) Diagnostic microbiology laboratory (R. C. Simkins, P. C. Brennan)

7) Necropsy laboratory (T. E. Fritz)

8) Histopathology laboratory (T. E. Fritz, P. H. Polk)

9) Surgical suite with inhalation anesthesiology equipment (C. M. Poole, W. G. Keenan)

10) Gnotobiotic isolators for germ-free technology (J. G. Linsley, L. O. Bibbs)

An important aspect of the success of any animal research program is the health of its animal population. To assure a supply of high quality animals, the Facility has concentrated on breeding its own healthy, disease-free animals. Most of the rodents, particularly mice, and all dogs are bred in the Facility. The rodent breeding is managed by L. O. Bibbs and Jane M. Angerman. The beagle breeding is supervised by Calvin M. Poole and William G. Keenan, who are also responsible for the clinical and surgical care of the dogs. The beagle colony has been a closed colony for more than 20 years, and extensive computerized records are maintained on all aspects of the colony, including reproduction, genetics, hematology, pathology, and disease incidence.

As important as the physical plant and its support facilities are the staff available to manage, monitor, treat, and evaluate the animals. The personnel provide a complete range of services to users of the experimental animals. Care of the animals is performed by a group of Animal Care Specialists, listed below, under the supervision of Durward D. Banister.

The Animal Care Specialists during 1979 were the following:

Claude C. Colegrove, Group Leader
Leo C. Farcus, Group Leader
Edward W. Jackson, Group Leader
William G. McDade, Jr., Group Leader
Earl R. Allen
Mose Burrell
Lucille E. Daley
Charles J. Fowler
Carrey R. Herringer
James L. Johns
Kenneth R. Muller
Cathleen L. Nelson
William O. Robinette
Richard M. Santarelli
Susan L. Santarelli
Leon L. Stewart
Diane M. Thomas
Joseph N. Wilson

COMPUTER SUPPORT FACILITY

F. S. Williamson, J. A. Blomquist, and C. A. Fox

Computer support is centered on the Remote Access Data Station (RADS), which is equipped with a 1000 lpm printer, 1000 cpm reader, and a 300 cps paper tape reader with 500-foot spools. The RADS is located in a data preparation room with four 029 key punch machines (two interpreting), a reproducer, storage vault for archival magnetic tapes, card files, and a 300 baud terminal for job status inquiry. An adjacent room provides work space for users, with a documentation library and consultant's office. This area includes Hewlett Packard graphics equipment--a 1200 baud HP2648A graphics display terminal and a 1200 baud HP7221A flat-bed pen plotter (four color).

Much software has been created to provide batch and interactive graphics, in large part using Argonne standard device-independent graphics.

The advent of the Conversational Monitor System (CMS) at Argonne in June 1979 launched a new effort to set up conversational computing and graphics software for our users. In particular, we have provided consultation and help for many users starting to use the new TELLAGRAF plotting package, and work is under way to support fully the BIMFILE record system on CMS in addition to OS/MVT batch.

Three standard data acquisition systems using cost-effective Digital Equipment Corporation LSI-11/2 microprocessors and recording on 4.5 M Byte tape cartridges have been assembled. Operating systems and custom software are created and tested on a PDP-11/20 using DOS. Tape cartridge data are converted to IBM-compatible tape on a PDP-11/03 system using RT-11.

An outgrowth of this data acquisition development was the rapid construction of a backup system for the Small Angle Neutron Scattering Instrument (H-2 beam) on the Intense Pulsed Neutron Source (ZING-P').

Approximately 50 users in the Division continue to depend on these facilities and the consultation on job management, programming, and data management that we provide.

RADIATION FACILITIES

G. L. Holmblad, J. L. Hulesch, J. E. Trier, and F. S. Williamson

The Divisional radiation facilities include a number of gamma, neutron, and X-ray radiation sources with accompanying areas for related equipment and preparing, handling, and servicing animals. These sources are described in detail below. There are five ^{60}Co irradiation facilities which provide a unique and versatile gamma-ray irradiation capability and a research reactor (JANUS), a source of fission-spectrum neutrons dedicated to biological research. The gamma-ray facilities are suitable for long-term studies in which animals can be maintained in the radiation field for duration of life or for acute brief or fractionated exposures. They provide a choice of exposure rates ranging from 0.004 to 3 x 10^4 R/minute. The mean energy of the JANUS neutrons is 0.85 MeV, and the available dose rates range between 0.002 to 80 rads/minute. The contamination from gamma radiation is remarkably low, between 3 and 4 percent of the total absorbed dose. X-ray facilities consist of two X-ray machines, one for experimental irradiations and one for clinical diagnostic purposes. Research using the Divisional radiation facilities is described principally in Sections 2, 3, and 11 of this report.

In addition to the radiation sources maintained in the Division for the irradiation of biological materials, two other neutron sources in the Chicago area are available to the staff for cell biology studies comparing neutron beams with different characteristics: neutrons from the linear accelerator at the Cancer Therapy Facility of the Fermi National Accelerator Laboratory ($\underline{p}^+ \rightarrow$ Be, mean energy 25 MeV) and cyclotron produced neutrons ($\underline{d}^+ \rightarrow$ Be, mean energy 3.6 MeV) at the Franklin McLean Research Institute, The University of Chicago.

^{60}Co Gamma Radiation

F-005 Gamma Room Radiation Field Dosimetry

Cavity configuration. Exposure rates from 1300 to 20,000 R/minute with ± 2% variation within volumes as large as 25 cubic centimeters.

Panoramic configuration. Exposure rates from 20 mR/minute to 10 R/minute with a worst case deviation of 7% from the mean within an arc large enough to contain 500 small rodents. Exposure rates as high as 50 R/minute can be achieved with up to 80 animals.

F-101 Gamma Room Radiation Field Dosimetry

Panoramic configuration. Exposure rates from 1 mR/minute to 50 mR/minute by varying distance and attenuation. Up to 500 small rodents may be irradiated simultaneously at four different expo-

sure rates with a worst case deviation of 7% from the mean. Animals are maintained in individual plastic cages, with food and water, on a frame with an N x 5 matrix. N varies from 4 to 8 depending on the frame distance from the source.

F-114 Gamma Room (Intermediate) Radiation Field Dosimetry

Panoramic Configuration. Exposure rates from 5 R/day to 40 R/day by varying distance and attentuation. Up to 52 dogs may be irradiated simultaneously at four different exposure rates.

X and Y Gamma Rooms Radiation Field Dosimetry

Panoramic configuration. In room X, exposure rates of 1.0 R/day and 2.5 R/day on two arcs, each of which can contain up to 50 individually caged dogs. In room Y, exposure rate of 0.4 R/day on one arc which can contain up to 100 individually caged dogs.

Fission-Spectrum Neutron Radiation

JANUS Reactor Radiation Field Dosimetry

Panoramic configuration. Dose rates from 0.002 to 25 rads/minute over a matrix of 400 mice with a worst case deviation of 8% from the mean. The gamma dose contribution is only 3-4% of the total absorbed dose.

Cavity configuration. A restricted room area near the converter plate may be used for irradiating small samples of cells at up to 65 rads/minute with good uniformity.

X-Radiation

General Electric Maxitron 250-kV X-ray machine, a general Divisional facility.

Westinghouse Flurodex "300" 130-kV X-ray unit equipped with a Machlett, Dynamax-40, rotating anode, dual focal spot tube. Used for clinical and diagnostic purposes for small animals and dogs.

ELECTRON MICROSCOPE CENTER

T. M. Seed and G. T. Chubb

The facility has approximately 2,600 square feet of working laboratory space, and includes two fully equipped photographic darkrooms, sectioning and autoradiographic facilities, five microscope cubicles, and four transmission electron microscopes and one Cambridge scanning electron microscope equipped with an X-ray energy dispersive analytical system. Ancillary specimen preparative equipment includes vacuum evaporators, freeze-drying and freeze-etching equipment, ultramicrotomes, and assorted photographic and light microscopic equipment.

The Electron Microscope Center and its personnel provide specialized electron microscope services to both the Division and the Laboratory as a whole. G. T. Chubb continues to maintain EM-related equipment and to provide needed technical assistance to all users of the Center. Active collaborative interaction between the Center and various Divisional research groups continues as, for example, in the research on cellular indicators of preclinical phases of leukemia, described in Section 2 of this report. Ultrastructural data were presented at various local, national, and international meetings. In addition, the Center has, through the active support of Argonne's Center for Educational Affairs and with Western Michigan University, established a technical training program for postgraduate individuals interested in pursuing a career in electron microscopy.

During 1979 there were some 15 researchers who made active use of the available facilities. They included 5 regular Argonne staff members, 1 postdoctoral appointee, 1 graduate student, and 3 visiting scientists. The remainder of the users were from other Argonne divisions, including Radiological and Environmental Research, Environmental Impact Studies, Chemistry, Physics, and Solid State Science.

17. Educational Activities

POSTGRADUATE TRAINING

During 1979, a total of 26 postdoctoral appointees and research associates contributed to the research programs of the Division. Five of these were new appointments in 1979, while eleven finished their assignments during the year.

The temporary appointees, their schools, and the staff members with whom they were affiliated were as follows:

Nadine N. Beales	University of Illinois at the Medical Center	C. A. Reilly
Laszlo Bodoni	Loyola University, Chicago	C. A. Reilly
Donna M. Buchholz	University of Illinois at the Medical Center	T. M. Seed
Franco M. Buonaguro	Instituto di Patologia Generale, Naples, Italy	M. M. Elkind
Anne L. Cahill	University of Michigan, Ann Arbor	C. F. Ehret
Bruce R. DeMark	University of Southern California	P. D. Klein
Pamela L. Derstine	Northwestern University	T. Matsushita
Jesse J. Edwards	University of Alabama, Birmingham	N. G. Anderson
Karen B. Ekelman	Ohio State University	C. Peraino
Michael L. Garriott	Purdue University	D. Grahn
Michael E. Ginevan*	University of Kansas	D. Grahn
Carol S. Giometti	University of Illinois at the Medical Center	N. G. Anderson
Viola M. Griego	Washington State University	R. B. Webb
Nelson D. Horseman	Louisiana State University, Baton Rouge	C. F. Ehret
Charles S. Irving	Weizmann Institute, Israel	P. D. Klein
Frederick R. Kirchner	University of Iowa	P. C. Brennan
George R. Lankas	University of Cincinnati	M. M. Elkind
Ellen H. Lau+	College of DuPage	Y. E. Rahman

*Now Assistant Statistician.

+Research Associate.

Hsien-Chi Niu	University of Missouri, Kansas City	P. D. Klein
Kanaiyalal R. Patel	University of South Dakota	Y. E. Rahman
Pepi Ross-Riveros	University of California, Berkeley	M. M. Elkind
Richard S. Rosenberg	University of Chicago	G. A. Sacher
Eung K. Ryu	Michigan Cancer Foundation, Detroit	M. MacCoss
Herbert M. Schwartz	Brandeis University	S. S. Danyluk
Frans Stellaard	University of Technology, The Netherlands	P. D. Klein
Fred J. Stevens	Michigan State University, East Lansing	M. Schiffer

In addition, there were seven Faculty Research Participation appointments, supported by the Argonne Center for Educational Affairs (CEA) and three Visiting Scientists. These appointments enable college and university faculty members to participate in the research activities of the Laboratory in order to broaden their perspectives for teaching and research on their home campuses. The names of the Faculty Research Participants and Visiting Scientists during 1979, their schools, and their staff sponsors were as follows:

William E. Boernke	Nebraska Wesleyan University	C. Peraino
Eugene W. McArdle	Northeastern Illinois University	C. F. Ehret
William F. Millington	Marquette University	T. M. Seed
George M. Mukunnemkeril	North Central College, Naperville	T. M. Seed
Daniel G. Oldfield	DePaul University	T. M. Seed
Nicolas Panagiotopoulos*	Nuclear Research Center, Athens, Greece	M. Schiffer
Shiva P. Singh	Alabama State University	T. E. O'Connor
S. Peter Spragg*	University of Birmingham, Birmingham, England	N. G. Anderson
Bartlett D. Whelton	Eastern Washington University	M. Bhattacharyya
Hiroshi Utsumi*	Kyoto University, Japan	M. M. Elkind

SUMMER RESEARCH INSTITUTE IN CELL BIOLOGY

Twelve students from eleven different universities were enrolled during the Summer of 1979 in this graduate level program offered by the Division of Biological and Medical Research in cooperation with the Argonne Center for Educational Affairs. Dr. Emerson W. Chan served as organizer and coordinator. The 12-week program featured a lecture series covering a variety of topics, including mechanisms of mutagenesis and carcinogenesis, assessment of effects of energy related pollutants on biological systems, techniques and applications of two dimensional electrophoresis and stable isotopic tracers, use of

*Visiting Scientist.

nucleoside analogues as chemotherapeutic agents, and U. S. energy issues and options. The lectures were given by Drs. P. C. Brennan, E. W. Chan, S. S. Danyluk, J. H. Edwards, D. Grahn, D. L. Hachey, H. E. Kubitschek, C. K. Lee, T. E. O'Connor, C. A. Reilly, G. A. Sacher, T. M. Seed, and R. B. Webb.

The lectures were supplemented by informal discussions and visits to laboratories as appropriate. Each student spent the remainder of his time working in a laboratory of a staff member.

The students, their schools, and their staff sponsors were as follows:

Cheryl Agris	Goucher College	P. D. Klein
Patricia Fields	Pennsylvania State University	H. E. Kubitschek
Douglas F. Fix	Indiana University Medical Center	R. B. Webb
Steven M. Holland	St. John's College, Maryland	T. E. O'Connor
Sophia S. Hom	Carleton College	C. K. Lee
David M. Kiser	Pennsylvania State University	G. A. Sacher
Nelson C. Labbe	North Adams State College	P. C. Brennan
Gregory G. Martin	University of San Diego	C. A. Reilly
Leo R. Parpart	Western Michigan University	T. M. Seed
Linda Ross	California State University, Northridge	N. G. Anderson
Rebecca R. Sheline	Bryn Mawr College	S. S. Danyluk
Gay-Ellen Stulp	Calvin College	E. W. Cahn

OTHER GRADUATE PROGRAMS

Four graduate students were Laboratory Graduate Participants working in the Division on research for their Ph.D. degrees in a program administered and suported by the Center for Educational Affairs. The Laboratory Graduate Participants, their schools, and their staff sponsors were as follows:

Barbara E. Amsler	University of Wisconsin, Milwaukee	E. W. Chan
Steven H. Gray	University of Illinois at the Medical Center	M. MacCoss
Bruce Hammer	Northwestern University	S. S. Danyluk
Kevin R. Rupprect	University of Notre Dame	E. W. Chan

A related program, called Thesis Parts, is also supported by the Center for Educational Affairs. It enables graduate students to perform pertinent parts of their research at Argonne. In 1979, one student held this appointment in the Division:

Roger G. Ulrich	Western Michigan University	T. M. Seed

In addition, eight graduate students carried out research in the Division as Resident Student Associates, supported directly by the Division unless otherwise noted:

John R. Baj	University of Illinois	D. Grahn
Stephen R. Gawne	University of Illinois	T. E. O'Connor
Sunny S. Kim	Northwestern University	S. S. Danyluk
E. Douglas Lewandowski	University of Chicago	T. E. Fritz
Gregory G. Martin	University of San Diego	T. E. O'Connor
William Obermeyer	University of Chicago	G. A. Sacher
Richard S. Skyer*	Rochester Institute of Technology	S. P. Stearner
Karen E. Willard	Virginia Polytechnic Institute and State University	N. G. Anderson

UNDERGRADUATE TRAINING

During 1979, a total of 24 college undergraduates received training in the Division of Biological and Medical Research through the CEA-sponsored Spring, Summer, and Fall Undergraduate Research Participation Programs. The students, their schools, and their staff supervisors are listed below:

Spring Program

Cheryl Agris	Goucher College	P. D. Klein
Rory F. Finn	College of St. Francis, Illinois	C. A. Reilly
Judith U. Hibbard	Illinois Benedictine College	T. Matsushita
Douglas S. McBain	Doane College	B. S. Hass
Mary A. Mueller	St. Mary's College, Minnesota	C. F. Ehret
Pamela M. Ogor	Southern Illinois University	C. G. Ehret
Diane L. Peterson	University of Wisconsin, Eau Claire	C. Peraino
Karen E. Schumann	Skidmore College	B. S. Hass
Robert W. Smith	Illinois Institute of Technology	M. MacCoss
Robert S. White	Northern Illinois University	M. MacCoss
Virginia J. Wight	Auburn University	C. A. Reilly

Summer Program

Emily E. Brooks	Harding College	B. S. Hass
Belinda A. Hayes	University of Illinois, Champaign	N. N. Beales
Judith U. Hibbard+	Illinois Benedictine College	B. S. Hass

*Sponsored by the Argonne Affirmative Action Program.

+Also participant in the Spring Undergraduate Research Participation program.

Merlon M. Hines	Mississippi Valley State University	C. S. Borso
Laurie E. Lambert	Williams College	M. Schiffer
Douglas S. McBain*	Doane College	B. S. Hass
Doriane Miller	Illinois Institute of Technology	T. E. O'Connor
Mary A. Mueller*	St. Mary's College, Minnesota	C. F. Ehret
Pamela M. Ogor*	Southern Illinois University	C. F. Ehret
Diane L. Peterson*	University of Wisconsin, Eau Claire	C. Peraino
Constance D. Shabazz	Stillman College	C. A. Reilly
Robert W. Smith*	Illinois Institute of Technology	M. MacCoss
Robert S. White*	Northern Illinois University	M. MacCoss
Phyllis Wright	Eckerd College	S. P. Stearner

Fall Program

Steven P. Binette	Carroll College, Montana	T. E. O'Connor
Frank R. Burns	Villanova University	C. Peraino
Lynn W. Ching	University of Hawaii, Hilo	C. F. Ehret
Karl M. Rogers	College of the Virgin Islands	Y. E. Rahman
Gary R. Schwartz	Emory University	C. F. Ehret
Carol A. Tiefenthal	Kalamazoo College	G. A. Sacher

JOINT ARGONNE-UNIVERSITY APPOINTMENTS

During 1979, 21 staff members held a total of 25 faculty appointments at universities in the Chicago area. These appointments usually comprise limited teaching activities at the graduate level, generally of a specialized nature, which involve regular contact with students. They have led to cosponsorship of graduate students and to collaborative research efforts with faculty members, some of which are described in this report.

The affiliations with Chicago area universities were as follows:

University of Chicago

Mortimer M. Elkind	Timothy E. O'Connor
Robert N. Feinstein	George A. Sacher
David L. Hachey	Warren K. Sinclair
Peter D. Klein	Patricia Szczepanik-Van Leeuwen

Loyola University

Maryka H. Bhattacharyya	Arthur Lindenbaum
Thomas E. Fritz	

*Also participant in the Spring Undergraduate Research Participation program.

Northern Illinois University

Maryka H. Bhattacharyya
Thomas E. Fritz
Douglas Grahn
Herbert E. Kubitschek
Carl Peraino
Yueh-Erh Rahman
Christopher A. Reilly, Jr.
Warren K. Sinclair
John F. Thomson
Robert B. Webb

Northwestern University School of Medicine

Peter D. Klein

University of Health Sciences/Chicago Medical School

Antun Han

University of Illinois at the Medical Center

Steven S. Danyluk

University of Notre Dame

Emerson W. Chan

TWELFTH ANNUAL AUA-ANL BIOLOGY SYMPOSIUM

The 1979 Symposium, "Environmental Carcinogenesis: Estimation and Mitigation of Risk," was held at Argonne National Laboratory on April 23-25, under the joint sponsorship of the Divisions of Biological and Medical Research, Environmental Impact Studies, and Radiological and Environmental Research and the Argonne Center for Educational Affairs. Dr. Carl Peraino of the Division of Biological and Medical Research was the the program chairman.

The program of the symposium was designed to introduce first and second year students to the subject. Sessions dealt with the theoretical basis of testing for environmental carcinogenic factors, current test systems, and mitigation of environmental carcinogenic risk. In all, there were 14 individual presentations. A feature of the symposium was a final round-table discussion among representatives of government regulatory agencies, industrial management, labor, and environemntal law. It dealt with the social implications of the realization that a significant proportion of human cancer arises from man-made environmental contaminants. In addition, a poster session, organized by Dr. Tatsuo Matsushita, was held in the evening to foster further interchange among Argonne staff and the visiting students and faculty members. It included 41 posters, 14 prepared by university attendees, the rest by Argonne staff and students.

The symposium was directed toward graduate students, primarily but not exclusively from member institutions of the Argonne Universities Association. Over 200 students and faculty members attended and participated in discussions.

18. DIVISIONAL SEMINARS DURING 1979

Through October 1979, the Division of Biological and Medical Research Seminar Committee consisted of A. Han, Chairman, M. MacCoss, Co-Chairman, L. S. Lombard, R. T. Lundy, C. Peraino, and T. M. Seed. For the rest of 1979 and the first half of 1980, the Committee consisted of M. MacCoss, Chairman, E. W. Chan, Co-Chairman, T. E. Fritz, A. Han, C. Peraino, and P. Szczepanik-Van Leeuwen.

The Distinguished ANL Scientist Lecture Series, initiated by the Division in 1977, was continued with two lectures by Divisional staff in 1979. The first was given by Peter D. Klein on January 11th, and was entitled "The Bioanalytical Center Presents: Bile Acids, Breath Hydrogen, and Body Water." The second was presented by M. M. Elkind on March 22nd, and was entitled "Damage and Repair, a Principal Duality in Radiation Biology."

The General Seminar Program for 1979 consisted of a combination of in-house and visiting speakers, selected on the basis of recommendations from the staff. In addition, a number of informal seminars in specialized subjects were held during the year and during the summer period when the general seminars were not scheduled; some of these informal talks are indicated by an asterisk in the following listing.

Dr. Thomas M. Seed, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
"Protracted Gamma Irradiation and Leukemia - What are the Relationships?"
January 4, 1979

Dr. Peter D. Klein, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
"The Bioanalytical Center Presents: Bile Acids, Breath Hydrogen, and Body Water"
January 11, 1979

Dr. Antun Han, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
"Radiation-Induced Neoplastic Transformation In Vitro"
February 1, 1979

Dr. Benno Schoenborn, Biology Division, Brookhaven National Laboratory, Upton, NY
"Neutron Scattering Techniques for Analysis of Biological Structures"
(Joint Seminar with the Solid State Division.)
February 6, 1979

Dr. Martin J. Murphy, Jr., Memorial Sloan-Kettering Cancer Center, NY
"*In Vitro* Aspects of Erythropoiesis"
February 8, 1979

Dr. G. Barry-Pierce, University of Colorado Medical Center, Denver, CO
"Differentiation in Cancer"
February 15, 1979

Dr. Gary S. Stein, University of Florida, Department of Biochemistry, Gainesville, FL
"Regulation of Histone Gene Expression in Human Cells"
February 22, 1979

Dr. Emerson Chan, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
"The Role of Retroviruses in ^{90}Sr-Induced Murine Osteosarcomas"
March 1, 1979

Dr. Neena Schwartz, Northwestern University, Evanston, IL
"The Role of the Male Mouse in Controlling Estrous Cyclicity: Hormonal Events"
March 8, 1979

Dr. Vernon R. Young, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, MA
"Whole Body Lysine Flux in Adults"
March 15, 1979

Dr. M. M. Elkind, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
"*Damage* and *Repair*, a Principal Duality in Radiation Biology"
March 22, 1979

Dr. Carlo M. Croce, The Wistar Institute, Philadelphia, PA
"Somatic Cell Hybrids to Study Cell Regulation"
March 29, 1979

Dr. S. Phyllis Stearner, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
"Radiation Effects on the Cardiovascular System"
April 5, 1979

Dr. P. O. P. Ts'o, School of Medicine and Public Health, The Johns Hopkins University, Baltimore, MD
"Basic Mechanism of Neoplastic Transformation as Related to the Establishment of a Biomedical Risk Assessment System"
April 12, 1979

Dr. Elliot D. Weitzman, Department of Neurology, Montefiore Hospital, New York, NY
"Studies of Man in Temporal Isolation: Sleep-Wake Cycles, Neuroendocrine and Body Temperature Measurements"
April 19, 1979

Dr. David A. Haugen, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
"Characterization of Microsomal Cytochrome P-450 Using Biphenyl as a Model Substrate"
May 3, 1979

Dr. Joseph Meites, Department of Physiology, Michigan State University, East Lansing, MI
"Relation of the Neuroendocrine System to Reproductive Decline During Aging"
May 10, 1979

Dr. Thomas H. Jukes, Division of Medical Physics, Space Science Laboratory, University of California, Berkeley, CA
"Some Nutritional Controversies: Carcinogens, Laetrile, and Health Foods"
May 15, 1979

Dr. Carl A. Keller, National Institute of Child Health and Human Development, Bethesda, MD
"Reproductive Efficiency as a Measure of Adverse Health Effects Due to Environmental Chemical Exposures"
May 17, 1979

Dr. Donald Olins, Oak Ridge National Laboratory, Oak Ridge, TN
"Conformational States of Histones and Nucleosomes"
May 25, 1979

*Dr. Michael L. Garriott, Purdue University, Purdue, IN
"Mutagenic Potential of Hyperthermia"
June 19, 1979

Dr. Wayne E. Magee, Division of Allied Health and Life Sciences, University of Texas, San Antonio, TX
"Liposomes as Delivery Systems for Nucleic Acids"
June 21, 1979

*Dr. Sankar P. Basu, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN
"Structure of a High Sulfur Containing Soybean Trypsin Inhibitor. Structure of Bulbocapnine Methiodide"
July 2, 1979

*Dr. David Hirst, Gray Laboratory, Mount Vernon Hospital, Northwood, Middlesex, England
"Some Effects of Radiation on the Vasculature of the Mouse Mesentery"
July 20, 1979

*Dr. Caleb Finch, University of Southern California, Los Angeles, CA
"Neurobiological Theory of Aging"
August 1, 1979

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*Informal seminar.

*Dr. T. Kameswar Rao, Oak Ridge National Laboratory, Oak Ridge, TN
"Use of Short-Term Genetic Testing for the Isolation Identification of Energy Related Pollutants"
August 16, 1979

*Ms. Patricia Sawzik, Department of Crystallography, University of Pittsburg, Pittsburg, PA
"Cholesterol Esters: Crystal and Mesophase Structures"
September 17, 1979

Dr. Ann Ganesan, Department of Biology, Stanford University, CA
"DNA Repair in Bacterial and Mammalian Cells"
September 20, 1979

Dr. E. Riklis, Nuclear Research Center, Beer Sheva, Negev, Israel
"Are We at Risk from Low-Level Radiation: DNA Repair Studies"
September 25, 1979

Dr. C. D. Lytle, Bureau of Radiological Health, Food and Drug Administration, Rockville, MD
"Radiation Enhanced Reactivation of Animal Viruses"
September 27, 1979

Dr. Malcolm MacCoss, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
"Synthetic Transformations and Preliminary Biological Evaluations of Selected Nucleoside Analogues"
October 4, 1979

Dr. Lawrence D. Aronson, Department of Medicine, Michigan State University, East Lansing, MI
"Recent Investigations of α_1-Antitrypsin"
October 11, 1979

Dr. Alàin Reinberg, Fondation A. de Rothschild, Laboratoire de Physiologie, Paris, France
"Chronobiological Approach of the Tolerance to Shift Work"
October 15, 1979

Dr. Paul C. Lauterbur, Department of Chemistry, State University of New York, Stony Brook, NY
"NMR in Spatial and Spectroscopic Dimensions: Zeugmatographic Imaging in Medicine and Elsewhere"
October 18, 1979

Dr. Leigh Anderson, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
"Review of the Molecular Anatomy Program"
November 1, 1979

*Informal seminar.

Dr. Michael B. Rabinowitz, Veterans Administration, Wadsworth Hospital Center, Los Angeles, CA
"Kinetic Analysis of Lead Metabolism in Healthy Humans"
November 8, 1979

Dr. Emerson Chan, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL
"Antiviral Properties of Thiol and Methyl Substituted Polyinosinic Acids"
November 29, 1979

Dr. Roger H. Kennett, Department of Human Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA
"The Genetics of Human Tumor-Associated Antigens Before and After Hybridomas"
December 6, 1979

Dr. Yves Fouron, Chembromed Ltd., University of Alberta, Edmonton, Alberta, Canada
"Some Applications of Carbohydrate Chemistry to Biological Problems - Present and Future Uses"
December 13, 1979

Dr. R. N. Tennant, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN
"Mechanisms of Induction and Control of RNA Tumor Virus Expression"
December 20, 1979

19. OUTSIDE TALKS BY DIVISIONAL STAFF DURING 1979

Anderson, N. G. "Genetic Implications of Two-Dimensional Protein Gene Product Mapping." Academy of Clinical Laboratory, Hanover, NH, May 31, 1979.

Anderson, N. G. "Monitoring Human Populations at Risk: Applications of Two-Dimensional Protein-Gene-Product Mapping." Third European Congress of Clinical Chemistry, Brighton, England, June 7, 1979.

Anderson, N. G. "Genetic Implications of Two-Dimensional Protein Gene Product Mapping." Cambridge University, Cambridge, England, June 14, 1979.

Anderson, N. G. "Monitoring Populations at Risk: Applications of Two-Dimensional Protein-Gene-Product Mapping." U.S./Japanese Workshop on Population Monitoring, University of Hawaii, East-West Center, Hawaii, July 16-23, 1979.

Anderson, N. G. "Two-Dimensional Mapping of Human Protein Gene Products in Health and Disease." Seventeenth Nordic Congress on Clinical Chemistry and Clinical Physiology, Oslo, Norway, August 3-11, 1979.

Anderson, N. G. "Two-Dimensional Mapping of Human Protein Gene Products in Health and Disease." XVII Nordiske Kongress, Klinisk Kjemi og Klinisk Fysiologi, Oslo, Norway, August 7-10, 1979.

Anderson, N. G. "Indexing Human Protein Gene Products." Division of Health Computer Sciences, University of Minnesota, November 14, 1979.

Anderson, N. L. "High-Resolution, Two-Dimensional Mapping of Human Gene Products." Cold Spring Harbor Laboratory, Long Island, NY, April 26, 1979.

Anderson, N. L. "High-Resolution, Two-Dimensional Mapping of Human Gene Products." Pasteur Institute, Paris, France, October 1, 1979.

Anderson, N. L. "High-Resolution, Two-Dimensional Mapping of Human Gene Products." Electrophoresis '79, Munich, Germany, October 15-17, 1979. (Poster Presentation)

Beales, N. N. "Rat Tracheal Ring Cultures Used for the Evaluation of Oncogenic Activity of Polycyclic Aromatic Hydrocarbons." Thirtieth Annual Meeting of the Tissue Culture Association, Seatle, WA, June 10-15, 1979.

Beales, N. N. "Tracheobronchial Explants for Studies of Human Carcinogenesis and Neoplasia." Invited participant in round table session, 30th Annual Meeting of the Tissue Culture Association, Seattle, WA, June 14, 1979.

Boye, E. "Double and Single Strand DNA Breakage and Repair in E. coli Measured in Superinfecting Phage λ DNA." Sixth International Congress of Radiation Research, Tokyo, Japan, May 13-19, 1979.

Brennan, P. C. "The Effect of Reaerosolized Fly Ash from an Atmospheric Fluidized Bed Combustor on Murine Alveolar Macrophages." Nineteenth Annual Hanford Life Sciences Symposium, Richland, WA, November 22-24, 1979.

Brown, M. S. "Far- and Near-Ultraviolet Radiation Effects on Strains of Escherichia coli." Seventh Annual Minority Biomedical Support Symposium, Atlanta, GA, April 15-18, 1979.

Brown, M. S. "Synergistic Interactions Between Near-UV and 405 nm Radiation in Escherichia coli." American Society for Microbiology, Los Angeles, CA, May 4-8, 1979, and Honolulu, HI, May 8-11, 1979.

Chan, E. W. "Biochemical and Biophysical Characterization of Two Murine Osteosarcoma Viruses, FBJ and FBR." American Society for Microbiology, Honolulu, HI, and Los Angeles, CA, May 4-11, 1979.

Danyluk, S. S. "Cross-Polarization, Magic-Angle NMR Studies of Biological Systems." Stereodynamics of Molecular Systems, State University of New York, Albany, NY, April 22-23, 1979.

Danyluk, S. S. "Nuclear Magnetic Resonance Studies of Nucleoside Conformational Properties." NATO Advanced Study Institute, Sogesta, Italy, May 7-14, 1979.

Danyluk, S. S. "Cross-Polarization Magic-Angle NMR." Seminar at Max-Planck Institute, Göttingen, Germany, May 16, 1979.

Danyluk, S. S. "NMR Studies of Nucleic Acid Conformational Properties." The 1979 Great Lakes Regional Meeting of the American Chemical Society, Rockford, IL, June 4-6, 1979.

Danyluk, S. S. "Aspects of Nucleic Acid Conformations." Seminar at Department of Chemistry, Rennselaer Polytechnic Institute, Troy, NY, November 19, 1979.

Edwards, J. J. "Two-Dimensional Electrophoresis Mapping of Human Erythrocyte Proteins." Electrophoresis '79, Munich, Germany, October 15-17, 1979. (Poster Presentation)

Ehret, C. F. "Circadian Clocks--How They Relate to Health and Mental Health." Didactic Group Dialogue, Forest Hospital Foundation, Des Plaines, IL, February 14, 1979.

Ehret, C. F. "The Importance of Circadian Factors in Nutrition and General Medicine." The Preventive Care Conference, Hinsdale Hospital, Hinsdale, IL, March 23, 1979.

Ehret, C. F. "The Role of Circadian Cybernetics in Human Bioengineering and Behavior Modifications." Illinois Institute of Technology, Chicago, IL, March 27, 1979.

Ehret, C. F. "Biological Time Constants and Levels of Organization." Fourth Conference of the International Society for the Study of Time, Alpbach, Austria, July 1-11, 1979.

Ehret, C. F. "Induction of Circadian Dyschronism and of Phase-Angle Differences by Dietary Phenobarbital during Light-Dark Entrainment in the Rat." The XIV International Conference of the International Society for Chronobiology, Hannover, West Germany, July 8-12, 1979.

Ehret, C. F. "The Innate and Genetic Basis of Circadian Rhythms." NATO Advanced Study Institute, Hannover, West Germany, July 13, 1979.

Ehret, C. F. "The Chronon Theory of Circadian Rhythm Control." NATO Advanced Study Institute, Hannover, West Germany, July 17, 1979.

Ehret, C. F. "Consideration of Diet in Alleviating Jet Lag." NATO Advanced Study Institute, Hannover, West Germany, July 21, 1979.

Ehret, C. F. "The Role of Circadian Rhythms in Health." Physicians Radio, August 23, 1979.

Ehret, C. F. "The Circadian Connection to Problems from Jet Lag to Biomass Production." International Paper Company Corporate Research Center, Tuxedo Park, NY, September 5, 1979.

Ehret, C. F. "Neurochemistry and the Circadian Chronotype." Anatomy Department, University of Nebraska Medical Center, Omaha, NB, November 28, 1979.

Ehret, C. F. "The Circadian Connection to Orthochronal Medicine." Sixth Annual University of Nebraska Medical Center Student Research Forum, Keynote Speaker, Omaha, NB, November 30, 1979.

Elkind, M. M. "DNA and Its Repair: Are They Involved in Cell Killing?" Loyola University Graduate School, Maywood, IL, February 13, 1979.

Elkind, M. M. "Molecules, Targets, and Cells in Radiation Damage and Repair." M. D. Anderson 32nd Annual Symposium on Fundamental Cancer Research, Houston, TX, February 27, 1979.

Elkind, M. M. "Radiation Damage and Repair in Cells and Molecules (DNA): Are They Related?" Department of Biology, Illinois Institute of Technology, Chicago, IL, March 23, 1979.

Elkind, M. M. "Cells, Targets, and Molecules in Radiation Damage and Repair." Harvard Medical School, Cambridge, MA, April 19, 1979.

Elkind, M. M. "Damage-Repair in Mammalian Cells: Comparative Processes in Ionizing and Nonionizing Radiation Lethality." Sixth International Congress of Radiation Research, Tokyo, Japan, May 13-19, 1979.

Elkind, M. M. "Sublethal Damage and Repair: Relevance to Radiobiology and Therapy." Helsinki University Central Hospital, Helsinki, Finland, May 28-June 2, 1979.

Elkind, M. M. "Combined Action of Chemicals and Radiation." Helsinki University Central Hospital, Helsinki, Finland, May 28-June 2, 1979.

Elkind, M. M. "Fundamental Questions in Combination Radiotherapy-Chemotherapy." Helsinki University Central Hospital, Helsinki, Finland, May 28-June 2, 1979.

Elkind, M. M. "Reoxygenation and Its Potential in Radiotherapy." Helsinki University Central Hospital, Helsinki, Finland, May 28-June 2, 1979.

Elkind, M. M. "Hyperthermia: Cytotoxic Action with and without Radiation." Helsinki University Central Hospital, Helsinki, Finland, May 28-June 2, 1979.

Elkind, M. M. "'Single-Hit' Damage and Neoplastic Transformation." Helsinki University Central Hospital, Helsinki, Finland, May 28-June 2, 1979.

Elkind, M. M. "Damage and Repair, Critical Processes in Radiobiology." Helsinki University Central Hospital, Helsinki, Finland, May 28-June 2, 1979.

Elkind, M. M. "Repair of Radiation Damage: Molecular and Cellular Processes Related to Survival and Transformation." Society's Risks and Benefits from Radiation Exposure, Rush-Presbyterian-St. Lukes Medical Center, Chicago, IL, October 24, 1979.

Elkind, M. M. "Neoplastic Transformation In Vitro: Are Repair Processes Involved?" Department of Radiology and Radiation Biology, Colorado State University, Fort Collins, CO, November 20, 1979.

Elkind, M. M. "Radiation-Induced Neoplastic Transformation In Vitro: Are Repair Processes Involved?" Department of Radiation Medicine, Massachusetts General Hospital, Boston, MA, November 26, 1979.

Finkel, M. P. "Bone Cancer Induction by Radiation and Virus." Symposium on The Cancer Problem, AAAS Meeting, Austin, TX, January 8, 1979.

Finkel, M. P. "The Role of Viruses in ^{90}Sr-Induced Murine Bone Sarcomas. I. Isolation of Bone-Sarcoma-Associated Viruses." Sixth International Congress of Radiation Research, Tokyo, Japan, May 13-19, 1979.

Fritz, T. E. "Late Effects of Protracted Whole-Body Irradiation." Harvard University, Boston, MA, February 21-23, 1979.

Fritz, T. E. "Biological and Medical Effects of Protracted Low-Level Whole-Body Irradiation." Annual Meeting of the European Late Effects Project Group, Ulm, West Germany, March 1, 1979.

Fritz, T. E. "Continuous Whole-Body Irradiation: Myelogenous Leukemia and Other Dose and Dose Rate Effects." Lawrence-Berkeley Laboratory, University of California, Berkeley, CA, April 19, 1979.

Fritz, T. E. "Effects of Long-Term, Low-Dose, Whole-Body Irradiation." Purdue University, West Lafayette, IN, November 1, 1979.

Fry, R. J. M. "Fission Neutron Induced Tumorigenesis." Sixth International Congress of Radiation Research, Tokyo, Japan, May 13-19, 1979.

Ginevan, M. E. "A Poisson Trials Approach to Interpopulation Comparisons of Cause of Death Data." Second Workshop on Health Surveillance around Point Sources of Pollution, Los Alamos Scientific Laboratory, Albuquerque, NM, January 22-24, 1979.

Ginevan, M. E. "An Inequality Involving the Binomial Distribution with an Application to Maximizing the Power of Certain Binomial Tests." Biometric Society Meeting, Washington, DC, August 13-16, 1979.

Ginevan, M. E. "Radiation Risk Analysis in Human Populations." Special Seminar, Division of Biological Sciences, University of Kansas, Lawrence, KS, October 29, 1979.

Giometti, C. S. "Mapping of Rabbit Skeletal Muscle Proteins by Two-Dimensional Electrophoresis." Sixty-third FASEB Meeting, Dallas, TX, April 6-10, 1979.

Giometti, C. S. "Two-Dimensional Electrophoresis of Human Saliva." Electrophoresis '79, Munich, Germany, October 15-17, 1979. (Poster Presentation)

Grahn, D. "Genetic Effects of Low Dose Neutron Irradiation." Tenth Annual Meeting of the Environmental Mutagen Society, New Orleans, LA, March 8-12, 1979.

Grahn, D. "Dominant Lethal Mutations and Chromosome Aberrations Induced in Male Mice by Incorporated ^{239}Pu and by External Fission Neutron and Gamma Irradiation." IAEA Symposium on Biological Implications of Radionuclides Released from Nuclear Industries, Vienna, March 26, 1979.

Grahn, D. "Biomedical Effects of Low Levels of Irradiation." Series on the Health Effects of Low Level Radiation Exposure, University of Cincinnati, Cincinnati, OH, May 17-18, 1979.

Grahn, D. "Radiation Hazards in the Urban Environment." Chicago Hospital Council's Program, Chicago, IL, June 28, 1979.

Grahn, D. "Biomedical Effects of Low Level Radiation Exposure." St. Louis University, St. Louis, MO, September 28, 1979.

Grahn, D. "Genetic Effects of Radiation." Northern Illinois University, Department of Biological Sciences, DeKalb, IL, October 29, 1979.

Gray, S. H. "Synthesis and Solution Conformations of Deoxynucleosidyl (3' → 5') Arabiononucleoside Monophosphates." Medicinal Chemistry Seminar, University of Illinois at the Medical Center, Chicago, IL, February 13, 1979.

Gray, S. H. "Synthesis and Solution Conformations of Deoxynucleosidyl (3' → 5') Arabiononucleoside Monophosphates." MIKI Meeting, University of Iowa, Iowa City, IA, May 3-5, 1979.

Griego, V. M. "Comparative Sensitivities of Bacillus Thuringiensis Spores to Various Wavelengths in the Near-UV-Visible Region." American Society for Microbiology, Los Angeles, CA, May 4-8, 1979.

Griego, V. M. "Differential Sensitivity of Mammalian Cells during Exponential Growth to Near-Ultraviolet Light." The American Society for Cell Biology, Toronto, Canada, November 6-8, 1979.

Griego, V. M. "The Effects of Near Ultraviolet Light on a Mammalian Cell System." Loyola University School of Medicine, Maywood, IL, November 29, 1979.

Hachey, D. L. "Use of Volatile Metal Chelates for Isotope Ratio Analysis of Biomedical Samples." Twenty-seventh Annual Conference on Mass Spectrometry and Allied Topics, Seattle, WA, June 3-8, 1979.

Hagan, M. P. "Recovery from BUdR/Near-UV Damage in Synchronized Cultures of Chinese Hamster Cells." Sixth International Congress of Radiation Research, Tokyo, Japan, May 13-19, 1979.

Han, A. "Radiation Responses of Cultured Mammalian Cells." Loyola University Graduate School, Chicago, IL, February 6, 1979.

Han, A. "Radiation Carcinogenesis; Cellular Aspect of Induction and Repair." Radiotherapeutic Service, Veterans Administration Hospital, Hines, IL, February 12, 1979.

Han, A. "Dependence of Neoplastic Transformation Upon Radiation Quality." Winter Meeting of the Midwest Chapter of American Association of Physicists in Medicine, Argonne National Laboratory, Argonne, IL, March 10, 1979.

Han, A. "The Effect of Radiation Quality and Repair Processes on the Incidence of Neoplastic Transformation In Vitro." Sixth International Congress of Radiation Research, Tokyo, Japan, May 13-19, 1979.

Han, A. "Repair of Radiation Damage in Mammalian Cells: Its Relevance to Environmental Effects." Conference on Health Effects of Energy Production, Chalk River, Ontario, Canada, September 12-14, 1979.

Han, A. "Damage and Repair in Mammalian Cells Following Neutron Radiation." Cancer Research Unit, Allegheny General Hospital, Pittsburgh, PA, October 4, 1979.

Han, A. "Radiobiological Concepts of Radiation Damage Induction and Repair in Mammalian Cells." Department of Physiology and Biophysics, Chicago Medical School, University of Health Sciences, Chicago, IL, November 29, 1979.

Hass, B. S. "Genetic Studies with Continuous Cultures." Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN, February 1, 1979.

Hass, B. S. "The Chemostat in the Absence of any Activating Agent Is a Quantitative Measuring Device of the Mutagenicity of Polycyclic Aromatic Hydrocarbons and Products from Fossil Fuel Energy Conversion Process Streams." Environmental Mutagen Society Meeting, New Orleans, LA, March 8-12, 1979.

Haugen, D. A. "Characterization of Liver Microsomal Cytochrome P-450-Dependent Monooxygenases." Illinois State Univesity, Normal, IL, December 6, 1979.

Horseman, N. D. "Corticosteroid Injections Phase-Shift the Circadian Thermoregulatory Rhythm of Rats." American Society of Zoologists, Tampa, FL, December 27-30, 1979.

Johnson, M. E. "Spin Label EPR Detection of Small Aggregate Formation by Sickle Hemoglobin Under Non-Gelling Conditions." Magnetic Resonance in Chemistry, Biology, and Physics, Argonne National Laboratory, Argonne, IL, June 24-28, 1979.

Kirchner, F. R. "Mammalian Responses to Exposure to the Total Diluted Effluent from Fluidized Bed Combustion of Coal." Nineteenth Annual Hanford Life Sciences, Symposium, Richland, WA, November 22-24, 1979.

Klein, P. D. "Biomedical Applications of Stable Isotope Ratio Measurements." Gordon Research Conference on Chemistry and Physics of Isotopes, Santa Barbara, CA, February 4-10, 1979.

Klein, P. D. "Gas Chromatography-Mass Spectrometry Resource Facilities for the Clinical Research Centers of NIH." CRC Directors' Meeting, Washington, DC, May 7, 1979.

Klein, P. D. "^{13}C Breath Tests: Components, Technology, and Comparative Costs." American Gastroenterological Association Meeting, New Orleans, LA, May 21-23, 1979.

Klein, P. D. "Recent Developments in Biomedical Applications of Stable Isotopes." Symposium on New Developments in GC, HPLC, and MS in Clinical Chemistry, Northwick Park Hospital, Middlesex, United Kingdom, June 1, 1979.

Klein, P. D. "^{13}C: A Universal and Ubiquitous Tracer." American Chemical Society Sectional Meeting, Greensboro, NC, October 9, 1979.

Klein, P. D. "Role of the Stable Isotope Laboratory in the Children's Nutrition Research Center." Baylor College of Medicine, Houston, TX, October 23, 1979.

Kubitschek, H. E. "DNA Replication, Lesions, Mutagenesis, and Carcinogenesis." Wheaton College, Wheaton, IL, January 19, 1979.

Kubitschek, H. E. "Mutagenicity of Coal Fly Ash Recovered from Power Plant Precipitators." Tenth Annual Meeting of Environmental Mutagen Society, New Orleans, LA, March 8-12, 1979.

Kubitschek, H. E. "Biological Activity of Effluents from Fluidized Bed Combustion of High Sulfur Coal." Society for Occupational and Environmental Health, The Park City Environmental Health Conference, Park City, UT, April 3-6, 1979.

Kubitschek, H. E. "Prokaryotic Mutagenesis Assays in the Detection of Environmental Carcinogens." Twelfth AUA-ANL Biology Symposium, Argonne, IL, April 23, 1979.

Kubitschek, H. E. "Action Spectrum for Growth Delay Induced by Near-Ultraviolet Light in *E. coli* B/r K Induced by Near-Ultraviolet Light." Seventh Annual Meeting of American Society of Photobiology, June 24-28, 1979.

Lee, C. K. "*In Vitro* Isolation and Characterization of Retroviruses from ^{90}Sr-Induced Murine Osteosarcomas." Thirtieth Annual Meeting of the Tissue Culture Association, Seattle, WA, June 11-14, 1979.

Ley, R. D. "The Induction of DNA Crosslinks and Erythema in Mouse Skin with 8-Methoxypsoralen (8-MOP) and Near Ultraviolet Light." Seventh Annual Meeting of American Society of Photobiology, June 24-28, 1979.

MacCoss, M. "The Synthesis, Characterization, and Preliminary Biological Evaluation of Membrane-Targeted Molecular Depots of Selected Nucleoside Analogues." American Chemical Society/ Chemical Society of Japan, Honolulu, HI, April 1-6, 1979.

Matsushita, T. "DNA Repair and Sister Chromatid Exchange in Mouse Myeloma Cells." Department of Microbiology and Immunology, University of Hawaii, Honolulu, HI, February 14, 1979.

Matsushita, T. "DNA Repair and Sister Chromatid Exchange in Mouse Myeloma Cells." Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO, March 26, 1979.

Matsushita, T. "Bacterial DNA Replication." Department of Biology, Wheaton College, Wheaton, IL, October 3, 1979.

Matsushita, T. "Sister Chromatid Exchange and DNA Damage in Mouse Myeloma Cells." Department of Microbiology, Loyola University, Stritch School of Medicine, Maywood, IL, November 16, 1979.

Nance, S. L. "Identification of Proteins in Two-Dimensional Patterns." Electrophoresis '79, Munich, Germany, October 15-17, 1979.

O'Connor, T. E. "Biochemical and Immunological Markers of Neoplasia." Twelfth AUA-ANL Biology Symposium, Argonne, IL, April 23, 1979.

O'Connor, T. E. "Biochemical and Immunological Markers of Neoplasia." National Cancer Institute, Bethesda, MD, June 25, 1979.

O'Connor, T. E. "Biochemical and Immunological Markers of Neoplasia." Mount Sinai Hospital, New York, June 26, 1979.

O'Connor, T. E. "Biochemical and Immunological Markers of Neoplasia." Roswell Park Cancer Memorial Institute, Buffalo, NY, September 5, 1979.

O'Connor, T. E. "Biochemical and Immunological Markers of Neoplasia." Purdue University, LaFayette, IN, September 25-26, 1979.

Peraino, C. "Multistage Hepatocarcinogenesis." McArdle Laboratory, University of Wisconsin, Madison, WI, March 13, 1979.

Peraino, C. "Drug-Induced Enhancement of Hepatic Tumorigenesis." Department of Biology, Notre Dame University, South Bend, IN, March 28, 1979.

Peraino, C. "Tumor Promotion in Rat Liver." Department of Biochemistry, Oregon State University, Corvallis, OR, May 3, 1979.

Peraino, C. "Rat Liver As an in Vivo Test System for Environmental Carcinogenic Factors." Twelfth AUA-ANL Biology Symposium, Argonne, IL, April 23, 1979.

Peraino, C. "Multistage Hepatocarcinogenesis." Department of Pathology, Northwestern University Medical Center, Evanston, IL, September 5, 1979.

Peraino, C. "Initiation and Promotion of Liver Tumorigenesis." National Cancer Institute, International Conference on Carcinogenic and Mutagenic N-Substituted Aryl Compounds, Bethesda, MD, November 7, 1979.

Rahman, Y. E. "Potential of Liposomes in Cancer Chemotherapy." Rush Medical School, Chicago, IL, July 13, 1979.

Rahman, Y. E. "Drug Targeting by Liposomes Varying in Glycolipids." Rush Medical School, Chicago, IL, July 13, 1979.

Rahman, Y. E. "Liposomes and Metal Poisoning." The 14th Harden Conference, Kent, England, September 11, 1979.

Rahman, Y. E. "In Vivo Cell Targeting by Liposomes Containing Glycolipids." Nineteenth Annual Meeting of the American Society of Cell Biology, Toronto, Ontario, Canada, November 5, 1979.

Reilly, C. A. "Viruses and Cancer." McMullan Memorial Lecture, Monmouth College, Monmouth, IL, May 2, 1979.

Reilly, C. A. "The Role of Retroviruses in Radiation-Induced Bone Sarcomas." Monmouth College, Monmouth, IL, May 3, 1979.

Reilly, C. A. "The Role of Viruses in ^{90}Sr-Induced Murine Bone Sarcomas. II. Tumor Induction Studies." Sixth International Congress of Radiation Research, Tokyo, Japan, May 13-19, 1979.

Reilly, C. A. "Viruses and Cancer." Western Michigan University, Kalamazoo, MI, December 13, 1979.

Ross-Riveros, P. "Synergistic Response of Rat Gliosarcoma Cells to Hyperthermia and X-Irradiation." Sixth International Congress of Radiation Research, Tokyo, Japan, May 13-19, 1979.

Russell, J. J. "The Microdistribution, Retention, and Autoradiography of ^{241}Am-citrate in Mouse Testis." Health Physics Society Annual Meeting, Philadelphia, PA, July 8-13, 1979.

Sacher, G. A. "Evolution of Longevity." Gordon Conference, Santa Barbara, CA, January 22, 1979.

Sacher, G. A. "What Evolved, Longevity or Aging?" Botany Department, California State University, Los Angeles, CA, January 24, 1979.

Sacher, G. A. "Longevity vs. Aging as the Target for Biogerontological Research." Wadsworth VA Hospital, Immunological Department, Los Angeles, CA, January 26, 1979.

Sacher, G. A. "Energy Metabolism and Thermoregulation in Old Age." ASHRAE Symposium, Philadelphia, PA, January 30, 1979.

Sacher, G. A. "Environmental and Intrinsic Factors in Aging." North Carolina Public Health Department, Chapel Hill, NC, February 26, 1979.

Sacher, G. A. "Import of Gerontological and Geriatric Education for the Future of our Society." Ohio State University, Akron, Ohio Network of Educational Consultants in Aging, Akron, OH, April 23, 1979.

Sacher, G. A. "Longevity and Aging: The Basic Duality of Biogerontology." North Wisconsin Madison Gerontology Center, Madison, WI, April 26, 1979.

Sacher, G. A. "Comparative vs. Ontogenetic Paradigms for Tests of the Intrinsic Mutagenesis Hypothesis of Aging." Conference on Structural Pathology in DNA and Aging, Frieburg, West Germany, May 16, 1979.

Sacher, G. A. "Comments on the History of Ideas Concerning the Evolution of Longevity." Conference on Structural Pathology in DNA and Aging, Frieburg, West Germany, May 19, 1979.

Sacher, G. A. "Genetics of Longevity and Aging." University of Chicago Committee on Human Development, Chicago, IL, May 30, 1979.

Sacher, G. A. "Animal Models for Aging Research." Washington State University, School of Veterinary Medicine, Pullman, WA, June 22, 1979.

Sacher, G. A. "The Role of Energy Metabolism and Motor Activity in the Aging Process." University of Illinois School of Public Health, Chicago, IL, September 19, 79.

Sacher, G. A. "The Role of SAGE vis-a-vis the National Organizations." State of New York Association for Gerontological Education, Albany, NY, October 19, 1979.

Sacher, G. A. "The Problem of Support for Research on the Effects of Energy-Related Environmental Pollutants on the Aging Process and the Aged." Presentation at Presidential Symposium on Energy and the Aged, Gerontological Society Annual Meeting, Washington, DC, November 26, 1979.

Sacher, G. A. "Energy and the Aged: A Challenge to the Quality of Life in a Time of Discussing Energy Availability." Gerontological Society Presidential Symposium, Washington, DC, November 28, 1979.

Sacher, G. A. "Genetics of Longevity and Constitution in Laboratory Mice and Their Hybrids." University of Colorado, Boulder Institute for Behavioral Genetics, Boulder, CO, December 14, 1979.

Schiffer, M. "Refinement of the Mcg Bence-Jones Protein Dimer: A Progress Report." American Crystallography Association, Symposium of Structure and Activity of Macromolecules, Boston, MA, August 12-19, 1979.

Schiffer, M. "3D Structure of IgG Molecule." University of Chicago, Chicago, IL, October 29, 1979.

Schiffer, M. "The Structure of Immunoglobulin Light Chains." Committee on Immunology and Pathobiology Training, University of Chicago, Chicago, IL, October 29, 1979.

Schwartz, H. M. "Conventional and Cross-Polarization, Magic-Angle ^{13}C NMR of Solid Biological Samples." Magnetic Resonance in Chemistry, Biology, and Physics, Argonne National Laboratory, Argonne, IL, June 24-28, 1979.

Schwartz, H. M. "^{17}O NMR of Nucleic Acid Bases." Annual Meeting of Midwest NMR Spectroscopists, Upjohn Company, Kalamazoo, MI, November 10, 1979.

Seed, T. M. "Electron Microscopy and Cell Biology." Parke-Davis Laboratory, Detroit, MI, February 6, 1979.

Seed, T. M. "Mechanisms of Leukemia Induction by Radiation." Department of Biology, DePaul University, Chicago, IL, February 16, 1979.

Seed, T. M. "Radiation-Induced Canine Leukemia." Department of Microbiology, Ohio State University, Columbus, OH, March 8, 1979.

Seed, T. M. "Preparation of Intact In Vitro Clones of Blood Forming Stem Cells for SEM." Scanning Electron Microscopy/1979, Washington, DC, April 16-20, 1979.

Seed, T. M. "Mechanisms of Radiation-Induced Leukemogenesis." School of Dentistry, Loyola University, Chicago, IL, April 24, 1979.

Seed, T. M. "Kinetics and Consequences of Intraerythrocytic Parasitism in Malaria." International Conference on Malaria and Babesiasis, Mexico City, Mexico, May 2, 1979.

Seed, T. M. "Induction of Myelogenous Leukemia by Protracted Gamma Irradiation." American Association of Physicists in Medicine, Midwest Chapter, Annual Meeting, Northwest Community Hospital, Arlington Heights, IL, May 19, 1979.

Sorensen, E. M. B. "Chemotherapeutic Alteration of Lead Excretion and Tissue Distribution in Mice." Tennessee Academy of Science Annual Meeting, Nashville, TN, November 16-17, 1979.

Stearner, S. P. "Long-term Cardiovascular Effects of Ionizing Radiations in Relation to Life-Span." AAAS Symposium, Houston, TX, January 3-8, 1979.

Stellaard, F. "Hyocholic Acid, an Unusual Bile Acid in Byler's Disease." American Association for the Study of Liver Diseases, Chicago, IL, November 7-8, 1979.

Stevens, F. J. "Heterogeneity of κI Light Chains." The 63rd FASEB Meeting, Dallas, TX, April 6-10, 1979.

Stevens, F. J. "Role of the Third Hypervariable Region in Self Association of κI-Immunoglobulin Light Chains." Mid-West Autumn Immunology Conference, Detroit, MI, November 4-6, 1979.

Szczepanik-Van Leeuwen, P. "Hyocholic Acid, an Unusual Bile Acid in Byler's Disease." American Association for the Study of Liver Diseases, Chicago, IL, November 7-8, 1979. (Poster Session)

Taylor, J. "Estimation of Two-Dimensional Electrophoresis Spot Intensities and Positions by Modeling." Electrophoresis '79, Munich, Germany, October 15-17, 1979.

Tollaksen, S. L. "Two-Dimensional Electrophoresis of Human Urinary Proteins in Health and Disease." Electrophoresis '79, Munich, Germany, October 15-17, 1979. (Poster Presentation)

Tolle, D. V. "Experimental Canine Leukemia and Related Hemoproliferative Diseases. Cytomorphology, Cytochemistry, Ultrastructure, and Pathology." Eighth Annual Conference of International Society for Experimental Hematology, Rotterdam, The Netherlands, August 21-24, 1979.

Tolle, D. V. "Comparative Hematopathology of Myeloproliferative (MPD) and Lymphoproliferative (LPD) Disease of the Beagle." Thirtieth Annual Session of the American Association for Laboratory Animal Science, Atlanta, GA, September 16-21, 1979.

Turner, M. A. "Interaction of Broad-Spectrum Near-UV and Far-UV (254) Radiation in Strains of Escherichia coli with Differing Repair Capabilities." American Society for Microbiology, Los Angeles, CA, May 4-8, 1979 and Honolulu, HI, May 8-11, 1979.

Turner, M. A. "Comparative Mutagenesis with Near and Far UV in Strains of Escherichia coli with Differing Repair Capabilities." University of Missouri, Columbia, MO, May 29-31, 1979.

Utsumi, H. "Radioresistance Induced by Caffeine in a Radiation Sensitive Clone of V79 Chinese Hamster Cells." Sixth International Congress of Radiation Research, Tokyo, Japan, May 13-19, 1979.

Utsumi, H. "Damage-Repair in Mammalian Cells: Comparative Processes in Ionizing and Nonionizing Radiation Lethality." Sixth International Congress of Radiation Research, Tokyo, Japan, May 13-19, 1979.

Webb, R. B. "Any Activating Agent is a Quantitative Measuring Device of the Mutagenicity of Polycyclic Aromatic Hydrocarbons and Products from Fossil Fuel Energy Conversion Process Streams." Tenth Annual Meeting, Environmental Mutagen Society, New Orleans, LA, March 8-12, 1979.

Willard, K. E. "The Effect of Human Urinary Protein Synthesis in Novikoff Hepatoma Cells." Meeting of Tissue Culture Association, Midwest Branch, Chicago, IL, April 8, 1979.

Willard, K. E. "Mapping of Chromosomal Proteins from Rat Liver and Novikoff Hepatoma Cells by Two-Dimensional Electrophoresis." Sixty-third FASEB Meeting, Dallas, TX, April 6-10, 1979.

Willard, K. E. "Protein-Gene Product Mapping." Microbiology Departmental Seminar, Virginia Polytechnic Institute and State University, Blacksburg, VA, May 2, 1979.

Willard, K. E. "Alterations of Two-Dimensional Electrophoretic Maps in Human Peripheral Blood Lymphocytes Induced by Concanavalin A." Electrophoresis '79, Munich, Germany, October 15-17, 1979. (Poster Presentation)

20. PUBLICATIONS APPEARING IN CALENDAR YEAR 1979

JOURNAL ARTICLES

Anderson, K. M., M. Rubenstein, and T. M. Seed. In-Vivo Expression of a C-Type RNA Virus in Rat Ventral Prostate Epithelial Cells. Biochem. Biophys. Res. Commun. 86, 402-406 (1979).

Anderson, N. G. Making Molecular Catalogues. Nature 278, 122-123 (1979).

Anderson, N. G., and N. L. Anderson. Molecular Anatomy. Behring Inst. Mitt. 63, 169-210 (1979).

Anderson, N. G., N. L. Anderson, and S. L. Tollaksen. Proteins of Human Urine. I. Concentration and Analysis by Two-Dimensional Electrophoresis. Clin. Chem. 25, 1199-1210 (1979).

Anderson, N. G., N. L. Anderson, S. L. Tollaksen, H. Hahn, F. Giere, and J. Edwards. Analytical Techniques for Cell Fractions. XXV. Concentration and Two-Dimensional Electrophoretic Analysis of Human Urinary Proteins. Anal. Biochem. 95, 48-61 (1979).

Anderson, N. L. The β and γ Cytoplasmic Actins are Differentially Thermostabilized by MgADP; γ Actin Binds MgADP More Strongly. Biochem. Biophys. Res. Commun. 89, 486-490 (1979).

Anderson, N. L., and N. G. Anderson. Microheterogeneity of Serum Transferrin, Haptoglobin and α_2HS Glycoprotein Examined by High Resolution Two-Dimensional Electrophoresis. Biochem. Biophys. Res. Commun. 88, 258-265 (1979).

Anderson, N. L., W. J. Eisler, and N. G. Anderson. Analytical Techniques for Cell Fractions. XXIII. A Stable Thermal Gradient Device for Heat Denaturation Studies on Proteins. Anal. Biochem. 91, 441-445 (1978).

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