SCHEDULE 189 403781

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory — RX-Biomedical & Environmental Research
Laboratory — Program

1. Contractor: Associated Universities, Inc. — Contract No.: AT(30-1)-16 — Task No.:

2. Project Title: Exposure to External and Internal Radiation Medical Studies of Marshall Islanders and Study of Radiation Effects on Intracellular Mechanisms — 189 No.: RX-4

3. Budget Activity No.: RX-01-01-(a)

4. Date Prepared: May 1973

5. Method of Reporting:
Scientific Meetings
BNL Monthly Letter to AEC
Scientific Journals

6. Working Location: Brookhaven National Laboratory

7. Person in Charge: R. A. Conard

Principal Investigator:
R. A. Conard
K. D. Knudsen

8. Project Term: Continuing — From: — To:

9. Man-Years:

Direct Man-Years	FY 1973	FY 1974	FY 1975
Scientific & Professional	2.0	3.0	3.0
Other	3.0	5.5	6.0
Guests & Res. Collaborators	1.0	3.0	3.0
Total	6.0	11.5	12.0

10. Costs (In Thousands of Dollars):

	FY 1973	FY 1974	FY 1975
Research Division	185	241	264
Hospital Division	29	70	77
Research Costs	214	311	341
Equipment Obligations	8	15	35

11. Reactor Concept:

The Medical Research Center
Brookhaven National Laboratory
Upton, L. I., New York

12. Materials:

REPOSITORY Records Holding Area Bldg 494
COLLECTION Proposals - Field Work
RX-4
BOX No. 8
FOLDER Field Work Proposals 1973

1179164

Exposure to External and Internal Radiation
Medical Studies of Marshall Islanders and Study of Radiation
Project Title: Effects on Intracellular Mechanisms RX-01-01-(a)

13. Publications:

Oh, Y. H., and Conard, R. A. Effect of aging on acetate incorporation in nuclei of lymphocytes stimulated with phytohemagglutinin. Life Sci. 11, 677-84 (1972). 16715

Oh, Y. H., and Conard, R. A. Further studies on mitogenic components of phaseolus vulgaris phytohemagglutinin: subunit structure. Arch. Biochem. Biophys. 152, 631-7 (1972). 16699

Oh, Y. H., and Conard, R. A. Effect of aging on histone acetylation of the normal and regenerating rat liver. Science (in press). 16939

Demoise, C. F., and Conard, R. A. Effects of age and radiation exposure on chromosomes in a Marshall Island population. J. Gerontol. 27, No. 2, 197-201 (1972). 17235

14. Scope:

A) 200 Word Summary:

The continuing primary objective is the determination of the life-time effects of fallout radiation on the people of the Marshall Islands who were accidentally exposed to radioactive fallout in 1954. Medical surveys are now conducted at six-month intervals. An unexposed Rongelap population is also examined for comparison. These surveys are carried out jointly by Brookhaven National Laboratory, under the auspices of the Atomic Energy Commission, and the Trust Territory, Department of Interior. The results of the surveys are of great importance in regard to the development of thyroid lesions, acute leukemia, and growth impairment in exposed children.

Research studies are carried on to elucidate the intracellular mechanisms triggered by mitogens in normal cultured human lymphocytes and to determine the defects in lymphocytes from patients with disorders of cellular immunity. A purified substance, isolated from phytohemagglutinin causes widespread transformation and cell division of lymphocytes in culture. Tagged with tritium, this molecule is used to study possible altered function of human lymphocytes in immunodeficient diseases, particularly chronic lymphocytic leukemia. Intracellular localization and action of the tagged mitogen is studied by autoradiography and by biochemical studies of subcellular fractions.

B) Supplement to 200 Word Summary:

The 19th year post-exposure surveys of the Marshall Islands have been completed. In addition to the 239 people originally exposed, over 300 unexposed Marshallese are examined for a "comparison population" to assess late effects of radiation from fallout. The development of thyroid neoplasms and growth impairment in exposed children indicates the need for examinations to be as complete as possible. In additon to routine physical, hematological and other laboratory examinations, the surveys involve special

(See Continuation Sheet)

14. Scope: (Cont'd.)

studies related to aging, malignancy, reproduction, body burdens, and evaluation of low levels of radionuclides in relation to a slightly contaminated environment. Thyroid patients are returned to the United States for complete hospital and laboratory studies and surgical treatment.

Altered function of the lymphocyte occurs: in diseases associated with immunologic deficiency such as chronic lymphocytic leukemia and auto-immune diseases; as the result of radiation exposure; and with the aging process. These conditions are characterized by reduced response of lymphocytes in culture to phytohemagglutinin (PHA), a potent mitogen. The intracellular changes associated with lymphocyte transformation by mitogens are poorly understood, particularly at the molecular level. Mitogens from PHA are purified by physico-chemical procedures. The "pure" molecules are labeled with tritium and fluorescent tags for study of intracellular localization and mechanism of action. Identification and characterization of the mitogenic determinants of the molecule will be an important long-range objective. Another objective is to make use of the virus-enhancing action of the mitogens in the search for a possible virus that might be associated with lymphocytic leukemias.

15. Relationship to Other Projects:

The studies of the exposed Marshallese are closely related to the Atomic Bomb Casualty Commission's (Dr. Darling) studies in Japan and to the studies of the 23 Japanese fishermen exposed at the same time as the Marshallese to fallout (Dr. T. Kumatori).

The lymphocyte studies are related to work reported in budget activities RX-03-01-(b), RX-03-02-(d), RX-01-02, and RX-01-03-(d). Related studies elsewhere include those of Congdon and his co-workers at Oak Ridge National Laboratory on transplantation immunology. Purification of phytohemagglutinin is of interest to Takahashi at the University of Minnesota, Rigas at the University of Oregon Medical School, Goldberg at the University of California, and Yachnin at the University of Chicago.

16. Technical Progress in FY 1973:

The March 1972 survey (18 years after exposure) was interrupted before completion by local Micronesian politicians. Upon request of the Congress of Micronesia, however, the survey was completed in September. During this survey leukopenia was observed in a 19 year old boy and two additional cases of thyroid nodularity were detected. In the U.S. acute myelogenous leukemia was diagnosed in the boy who died while under treatment. The two individuals with thyroid nodularity were operated on revealing a papillary carcinoma in the 19-year-old girl (the first case among the children receiving only 69 rad of whole-body radiation, about 500 rad to the thyroid including radioiodines) and benign adenomas in the 29-year-old female exposed to 175 rad (thyroid dose of about 500 rad including radioiodines). Growth in these girls had been

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

normal and the prognosis in both cases is excellent.

A total of 23 exposed Rongelap people have developed thyroid abnormalities since 1963. When the development of thyroid abnormalities was first observed, it became apparent that growth retardation noted in some of the children was related to thyroid hormone deficiency from injury to that gland. The Rongelap exposed population were then placed on thyroid hormone treatment. It appears that this treatment has been beneficial with regard to improvement in growth in some of the children, though it is impossible to evaluate what prophylactic effect this treatment may have on the incidence of further nodularity or carcinoma of the gland.

During the year the three previously reported cases of thyroid malignancy among the Rongelap people were brought to Tripler Army Hospital in Hawaii for evaluation. One case showed no positive findings and was returned home. The other two cases showed questionable areas with the thyroid scan. They were brought to the United States for surgical exploration; no extension of the malignancy was found.

Since August 1972, Dr. Knud Knudsen from this laboratory, has been stationed in the Marshall Islands. He is carrying out extensive health care services for the Rongelap and Utirik peoples as well as checking on the thyroid treatment program. This service is appreciated by the Marshallese and is of great value in our assessment of the medical status of the people.

Without a competent biochemist to replace Dr. Oh, the anticipated progress on studies of mitogenic molecular subunits has been curtailed.

17. Expected Results in FY 1974:

The September survey included two medical observers from Japan, one from England and one from the Public Health Service. Their reports have been presented to the Congress of Micronesia. It is believed the reports are favorable and should dispel the false accusations made by the Congressmen about the surveys. In view of the seriousness of the leukemia and thyroid developments in the Marshallese, it is anticipated that the annual examinations will continue with hematological examinations every six months.

In the lymphocytic studies several objectives will be pursued during the coming year if funds are available. Methods for electron microscopic autoradiographic localization of the site of action of labeled mitogens as a function of time will be perfected. The mechanism of action of the mitogens will be investigated by a variety of studies: (1) interaction, (complexing) of the mitogens with various cellular elements; (2) correlation of the sites of action of the mitogen with changes in RNA, DNA and protein synthesis by

(See Continuation Sheet)

17. Expected Results in FY 1974: (Cont'd.)

biochemical and histo-chemical studies; (3) metabolic studies of separated purified organelle fractions by direct stimulation of separated organelles with the mitogen; (4) identification of the nuclear status of the mitogen and its complexes by chromatographic techniques.

In vivo studies on the mitogenic action of the purified molecules on the lymphatic system will be initiated in rabbits. The development of a specific antibody against the mitogen will be complexed with the tagged mitogen to study possible membrane localization and the effects of mitogenic action.

18. Expected Results in FY 1975:

Careful screening of the Marshallese populations for development of malignancy, particularly of the blood and thyroid gland and the development of other degenerative diseases will be continued on a long-term basis. Upon return of the Bikini and Eniwetok people to their home islands, it is anticipated that whole-body gamma spectrographic analyses will be carried out on these island groups as well as on the Rongelap people again.

Upon completion of the year that Dr. Knudsen has agreed to stay on the islands, it is hoped to obtain the services of another physician who will carry out these important supplementary health service functions. The services of such a physician are extremely valuable in putting the thyroid treatment program on a firm basis and in documenting health statistics on the Marshallese.

Studies will continue on localization and mechanism of action of the purified mitogen, induction of cellular transformation, and cell division in normal and leukemic lymphocytes. The in vivo use of the purified mitogens in animal studies will be of great interest for possible therapeutic considerations in view of the greatly reduced antigenicity of the purified mitogen. It is hoped that the basic defects in chronic lymphocytic leukemia and other disorders of immunologic competence will become apparent in the ensuing years.

19. Description and Explanation of Major Materials, Equipment, and Subcontract Items:

FY 1975 Capital Equipment:

Miscellaneous equipment for conducting the field surveys and for processing specimens collected for laboratory analyses at BNL is estimated at $10,000. A quarter share ($25,000) of the automatic autoradiographic grain counter and cyto-analyzer described in Section 19 of RX-01-03-(d) will be charged to this activity because of use of the equipment in cell studies.

(See Continuation Sheet)

Project Title: Exposure to External and Internal Radiation Medical Studies of Marshall Islanders and Study of Radiation Effects on Intracellular Mechanisms RX-01-01-(a)

20. Proposed Obligations for Related Construction Projects:

None

(See Continuation Sheet)

Project Title: Exposure to External and Internal Radiation Medical Studies of Marshall Islanders and Study of Radiation Effects on Intracellular Mechanisms RX-01-01-(a)

Reiteration of Assurance Statement
on
Investigation Involving Human Subjects
Including Clinical Research

The Hospital of the Medical Research Center, Brookhaven National Laboratory, complies with the policy for the protection of human subjects participating in activities supported directly or indirectly by grants or contracts from the Department of Health, Education, and Welfare and from the Atomic Energy Commission. In fulfillment of its assurance, this institution has established a standing committee to review investigations involving human subjects, including clinical research, and said committee is charged with responsibilities to determine for each proposed study that:

the rights and welfare of each patient will be adequately protected,
that the potential benefits outweigh the risks, and
that an informed consent will be obtained from each patient.

The committee, appointed by the Director, Brookhaven National Laboratory, provides for independent judgment of its members, and alternates are provided to serve when there may be conflict of interest. This institution encourages constructive communication between the committee and principal investigators on the safeguarding of the rights and welfare of subjects; will provide the required care for persons injured as a result of participation; and maintains complete documentation of proposals and reviews. Approved investigations are reviewed by the committee at least annually and the institution periodically reviews its practices and procedures to assure they are consistent with the policies published by the Department of Health, Education, and Welfare.

As of March 7, 1973, the Institutional Review Committee for review of Medical Department research activities involving human subjects is composed of:

J. S. Robertson, M.D., Chairman
G. C. Cotzias, M.D., Alt. Chairman
S. H. Cohn
E. A. Popenoe, Alt. for Dr. Cohn
G. Chikkappa, M.D.
H. Connell
R. A. Love, M.D.
G. Price
N. P. Rathvon, Jr.
D. N. Slatkin, M.D., Alternate
A. P. Wolf, Alternate
L. K. Dahl, M.D. - Clinical Consultant
W. A. Finn - Administrative Consultant
C. W. Flood - Health Physics Consultant

Signature: Eugene P Cronkite

Title: Chairman, Medical Department

Date: March 7, 1973

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory — Laboratory

RX-Biomedical & Environmental Research — Program

1. Contractor: Associated Universities, Inc.
Contract No.: AT(30-1)-16
Task No.:

2. Project Title: Exposure to External and Internal Radiation. In Vivo Measurement of Radionuclides in Man; Body Burden and Kinetic Factors. Computer Applications
189 No.: RX-11

3. Budget Activity No.: RX-01-01-(b)

4. Date Prepared: May 1973

5. Method of Reporting:
Scientific Meetings
BNL Monthly Letter to AEC
Scientific Journals

6. Working Location: Brookhaven National Laboratory

7. Person in Charge:
J. S. Robertson
S. H. Cohn
Principal Investigator:
J. S. Robertson
S. H. Cohn

8. Project Term: Continuing
From: To:

9. Man-Years:

Direct Man-Years	FY 1973	FY 1974	FY 1975
Scientific & Professional	6.5	6.5	6.5
Other	8.5	11.0	10.5
Guests & Res. Collaborators	1.5	3.0	3.0
Total	16.5	20.5	20.0

10. Costs (In Thousands of Dollars):

	FY 1973	FY 1974	FY 1975
Research Division	369	400	422
Hospital Division	69	91	108
Research Costs	438	491	530
Equipment Obligations	14	54	44

11. Reactor Concept:

12. Materials:

Project Title: Exposure to External and Internal Radiation
In Vivo Measurement of Radionuclides in Man; Body Burden and Kinetic Factors, Computer Applications RX-01-01-(b)

13. Publications:

Cohn, S. H., Cinque, T. J., Dombrowski, C. S., and Letteri, J. M. Determination of body composition by neutron activation analysis in patient with renal failure. J. Lab. Clin. Med. 79, No. 6, 978-94 (1972). 16004

Dombrowski, C. S., Wallach, S., Shukla, K. K., and Cohn, S. H. Determination of whole body magnesium by in vivo neutron activation. Intern. J. Radiation Isotopes (in press). 16077

Cohn, S. H., Shukla, K. K., Dombrowski, C. S., and Fairchild, R. G. Design and calibration of a "broad-beam" 238-Pu, Be neutron source for total-body neutron activation analysis. J. Nucl. Med. 13, No. 7, 487-92 (1972). 16442

Aloia, J. F., Roginsky, M. S., Jowsey, J., Dombrowski, C. S., Shukla, K. K., and Cohn, S. H. Skeletal metabolism and body composition in acromegaly. J. Clin. Endocrinol. Metab. 35, No. 4, 543-51 (1972). 16456

Wallach, S., Aloia, J., and Cohn, S. Treatment of osteoporosis with calcitonin. Seminars in Drug Treatment, 2, No. 1, 21-5 (1972). 16599

Cohn, S. H., Fairchild, R. G., and Shukla, K. K. Neutron sources, energy, flux density and moderation in total-body neutron activation analysis. Presented at the IAEA Panel on In-Vivo Activation Analysis, Vienna, April, 1972. 16655

Cohn, S. H., Fairchild, R. G., and Shukla, K. K. Comparison of techniques for the total-body neutron activation analysis of calcium in man. Presented at the IAEA Panel on In-Vivo Activation Analysis, Vienna, April, 1972. 16659

Shore, F. J., Robertson, J. S., and Bateman, J. L. Childhood cancer following obstetric radiography. Lancet (in press). 16664

Dombrowski, C. S., Wallach, S., Shukla, K. K., and Cohn, S. H. Radioisotope determination of whole body magnesium by in vivo neutron activation. Presented at the First International Symposium on Magnesium Deficit in Human Pathology, Vittel, France, May, 1971. 16919

Shukla, K. K., Ellis, K. J., Dombrowski, C. S., and Cohn, S. H. Physiological variation of total body potassium in man. Am. J. Physiol. (in press). 16921

[redacted]kla, K. K., Dombrowski, C. S., and Cohn, S. H. Fallout ^{137}Cs levels in man over a twelve year period. Nature (in press). 16922

Collipp, P. J., Curti, V., Thomas, J., Sharma, R. K., Maddaiah, V. T., and Cohn, S. H. Body composition changes in children receiving human growth hormone. Metab. (in press). 16963

(See Continuation Sheet)

1179172

Project Title: Exposure to External and Internal Radiation
In Vivo Measurement of Radionuclides in Man; Body Burden and Kinetic Factors, Computer Applications RX-01-01-(b)

13. Publications: (Cont'd.)

Cohn, S. H. Calcium homeostasis: The hard facts about soft bones. BNL Lecture Series, No. 109, March 1972. 50345

Cohn, S. H., Roginsky, M. S., Aloia, J. F., Ellis, K. J., and Shukla, K. K. Alteration in elemental body composition in thyroid disorders. J. Clin. Endocrinol. Metab. (in press). 17179

Cohn, S. H., Roginsky, M. S., Aloia, J. F., Ellis, K. J., and Shukla, K.K. Alterations in skeletal mass in endocrine dysfunctions as determined by total-body neutron activation analysis. Presented at the 9th European Symposium on Calcified Tissues, Baden, Austria, October, 1972. 17180

Cohn, S. H., Shukla, K. K., and Ellis, K. J. A multivariate predictor for total body calcium in man. Am. J. Physiol. (in press). 17234

Robertson, J. S., Marr, R. B., Rosenblum, M., Radeka, V., and Yamamoto, Y. L. 32-crystal positron transverse section detector. Presented at the Proceedings of the Symposium on Radionuclide Tomography, New York, September, 1972. 1723

Cohn, S. H., Roginsky, M. S., Aloia, J. F., Ellis, K. J., and Shukla, K. K. Alterations in skeletal calcium and phosphoids in dysfunction of the parathyroids. J. Clin. Endocrinol. Metab. (in press). 17294

14. Scope:

A) 200 Word Summary:

A long-rang objective of this program is the elucidation of the control of calcium metabolism, particularly in those disorders characterized by losses of calcium from the skeleton (osteoporosis) and in osteodystrophy associated with chronic hemodialysis. Mathematical models have been developed to quantitate Ca metabolism and to provide a reference for measurement of subtle changes in the metabolic parameters resulting from therapeutic efforts. Kinetic tracer studies employ Ca-47 to provide the data for compartmental analysis along with data from whole-body counting and in vivo neutron activation analysis of whole-body calcium. A second objective is the study of the shape of the radiation dose response curve. The shape of this curve at levels near the natural background dose rate is of interest regarding the role of natural background radiation in producing the normal mutation rate.

[redacted]trumentation development for scanning and image processing as well as associated computer programming are proposed as general objectives in this program. The work described is, in general, supportive of projects in other budget categories of the Medical Research Program.

B) Supplement to 200 Word Summary:

An essential requirement for the study of calcium metabolism was the

(See Continuation Sheet)

1179173

14. Scope: (Cont'd.)

development of a method for absolute measurement of internally deposited radionuclides using a whole-body counting technique. In addition, the technique of in vivo neutron activation analysis was developed in order to measure the rate of loss of Ca from the body in the numerous skeletal disorders under study. The paramount need for the in vivo measurement of absolute levels of internally deposited radionuclides occurs in the diagnosis of cases of accidental intake of radionuclides. It is also essential in numerous clinical studies. The main effort in the whole-body counting program has been directed toward minimizing the variation in counting efficiency due to spatial distribution of internally deposited radionuclides and changes in their energy absorption as a function of body weight and habitus.

A number of clinical studies which previously had not been amenable to investigation because of the necessity of absolute measurements or spatial localization of radionuclides have been conducted. These studies include: measurement of absolute levels of whole-body potassium and determination of its spatial distribution in the body; measurement of lean-body mass in grossly obese children, based on measurement of K-40; measurement of lean-body mass in growth-deficient children undergoing therapy with growth hormone; measurement of levels of Cs-137 and K-40 in BNL staff members as a function of time and sex; and calibration procedures for absolute measurements and computer programs associated with such measurements. Anti-coincidence circuitry is available in the whole-body counter and its use is being explored with administered positron emitters which would improve the spatial localization capability of the counting system.

Besides determining absolute levels of calcium in the body, levels of sodium, chlorine and phosphorus have been measured by neutron activation with the aim of developing diagnostic techniques as well as obtaining basic information on disease processes. Of the ten most abundant elements in the body, six have been measured and the possibilities of measuring hydrogen, carbon and oxygen are explored.

Effort has been made to improve the accuracy of the in vivo neutron activation technique by improving the uniformity of thermal neutron irradiatio flux density in the irradiated subjects. Activation is now performed with a unique Pu-238, Be neutron irradiation facility rather than with the 14 MeV neutron generator originally employed. The reduced dose to the patient, together with the inherent simplicity of operation of the PuBe neutron source, makes this technique the most desirable for total body neutron activation in medical diagnosis and research. (Cohn)

Data published by Oftedal indicate that for some end points very low doses of radiation may be more effective than would be predicted by extrapolation from high doses. It is proposed that experiments be conducted under conditions of less than natural background in order to define the response

(See Continuation Sheet)

14. Scope: (Cont'd.)

curve between zero and the natural dose rate. Because at these levels the events of interest are very infrequent, it is necessary to work with bacteria or with tissue cultures to obtain statistically significant data.

New research problems may require intensive work on computer programming for an initial period of time. Once the computer program has been developed to the point of doing the basic job requested, its use in data processing may become a routine part of a sequence of operations. Further refinements of the program may be spread out over a longer period of time. In these applications the data processing is not an independent end point but is imbedded in other goals. Similarly the development and maintenance of special radiation detection instrumentation usually depends on requirements arising out of other studies.

The physical facilities involved include the medical computing facility, the whole-body counter, the PuBe in vivo activation facility, the central counting rooms, a multidetector positron coincidence system and an electronics repair shop. Some special equipment is associated with the electron paramagnetic resonance (EPR) system, the Nuclear Medicine Laboratory, and with other data acquisition devices in the Medical Department. (Robertson)

15. Relationship to Other Projects:

Related kinetic studies of calcium metabolism have been performed by Bauer, Hospital for Special Surgery and Bronner, University of Louisville. Similar mathematical models have been used by Pak, NIH. As previously noted however, the conceptual basis of the work at BNL differs from that of the other investigators. Further, the use of sophisticated computer programs makes it possible to measure small changes in calcium metabolism not discernible by use of most other techniques.

Considerable effort has been devoted to minimizing the variations in counting efficiency in whole-body counting by Dudley, International Atomic Energy Agency; Marinelli, Argonne National Laboratory; Naversten, Lund, and Genna, Massachusetts Institute of Technology. The BNL counter is the only known system which can correct simultaneously for both the spatial distribution and energy absorption of radionuclides by the body.

Representative groups working on the same clinical studies are those of [redacted], Laurentian University of Sudbury, Canada, studying treatment of ure[redacted] patients with Vitamin D analogues to combat osteodystrophic changes; Hioco and Bordier, Paris, France on effect of synthetic calcitonin on calcium metabolism in osteoporosis; and Heaney, Creighton University Medical School, on skeletal response to growth hormone. Boddy, Scottish Universities

(See Continuation Sheet)

15. Relationship to Other Projects: (Cont'd.)

Research Reactor Centre, Glasgow; Remenchik, Argonne National Laboratory; Forbes, University of Rochester; and Burkinshaw, University of Leeds, have all measured whole-body potassium with high precision. Two groups are presently involved in total body neutron activation analysis (TBNAA) for calcium measurement: Chamberlain, University of Birmingham, England; and Nelp-Palmer, University of Washington.

The current proposal on minimal radiation involves lower doses of radiation than have been involved in other known experiments.

Related work on the positron coincidence imaging method is actively pursued at the Massachusetts General Hospital, Boston, by Dr. Gordon Brownell's group and at McGill University, Montreal, by Dr. Yamamoto. This is further related to the problems of tomography, on which a number of other approaches were discussed at a recent symposium, the proceedings of which are to be published.

16. Technical Progress in FY 1973:

Many studies involve neutron activation analysis and calcium kinetics. In the study on the effect of the administration of synthetic calcitonin together with dietary calcium and phosphorus supplements, the period was extended to 1-2 years and the number of osteoporotic patients was increased to 35 to include some administered human growth hormone rather than calcitonin. Following calcitonin administration clinical improvement was evidenced by a decrease in back pain and a cessation of spontaneous fractures as reported last year. The results following human growth hormone administration await completion of six months of therapy.

In the study on renal osteodystrophy, in vivo neutron activation analysis and whole-body counting techniques were used to measure the body composition in an additional 30 with results confirming those reported last year. In collaboration with Dr. Pierson, St. Lukes Hospital, New York, a serial TBNAA study of the changes in body composition and calcium metabolism both pre- and post-transplantation was initiated for 12 patients scheduled to receive kidney transplants. In the study on effect of calcitonin on Paget's disease initiated by Dr. Wallach, Downstate Medical School, Brooklyn, 12 patients have now been followed for one year while receiving calcitonin treatment. All of these patients show a higher than normal level of body calcium along with a greatly increased bone turnover rate.

The continuing studies on endocrine dysfunction are conducted to quantitate the changes in levels of calcium, sodium, chlorine, and phosphorus in patients with disorders of the thyroid and parathyroid glands as well as in acromegaly, diabetes, and Cushing's disease. These studies are carried on in collaboration with Drs. Roginsky and Aloia of the Nassau County Medical Center, New York.

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

TBNAA reveals a decrease in total body calcium in subjects exhibiting the condition of hyperthyroidism. With respect to an estimated normal total body calcium (Ca_E) for the individual based on height, sex, and potassium level, a mean decrease to 90.6% was observed. The empirical value of Ca_E uses body potassium as a parameter, as it is an index of lean body mass. The relationship derived empirically from a study of 9 normal males is $Ca_E = 54.5\sqrt{K}\ H$ and from a study of 5 normal females is $Ca_E = 57.0\ \sqrt{K}\ H$; where Ca and K are expressed in grams and height (H) in centimeters. The P/Ca ratio in hyperthyroid patients was slightly higher than the 0.55 value observed in normals. Treatment of thyrotoxicosis by drug therapy, by surgery or by administration of I-131 leads to an increase in the mean Ca level at nine months following therapy. A concomitant decrease in the mean P/Ca ratio was observed in these patients.

Loss of bone mass in thyrotoxicosis has generally been associated with increased bone resorption and a negative calcium balance. Changes in bone are most probably not mediated via alterations of parathormone (PTH) or calcitonin but rather are the result of the action of the thyroid hormone on the osteoclasts.

Similar analysis of hypothyroid patients indicated a mean calcium value of 93.5% of their expected value. The mean P/Ca ratio was also lower than in normal subjects. At nine months following the institution of maintenance therapy in three patients, the mean calcium decreased to 88% of the total body calcium. The therapy also resulted in an increase in the mean P/Ca ratio. Decreased urinary calcium excretion and variable phosphorus excretion has been reported for hypothyroid patients. Additionally, kinetic studies have revealed a decrease in bone turnover.

In parathyroid studies, hyperparathyroid patients exhibit a decrease in calcium to 89% of the expected value. A low mean P/Ca ratio was observed, consistent with evidence of a loss of phosphorus. This condition may result from the inhibition of proximal tubular resorption responding to an increase in the PTH level. Nine months following the initiation of a course of treatment, a further decrease in total calcium was observed in two of the three patients treated. PTH acts directly on bone as well as affecting the functioning of the kidneys and the absorption in the gastrointestinal tract. The finding of decreased total calcium is consistent with densitometric and radiographic evidence of demineralization in hyperparathyroid patients. A decrease in bone mass often precipitates, via the body's homeostatic mechanism, a compensatory increase in the bone accretion rate. Hence, the metabolic picture may vary considerably in the course of this disorder.

Hypoparathyroid patients exhibited an increased body calcium, in terms of the expected value. For example, one patient with idiopathic hypoparathyroidism showed an increase to 170% of the total-body calcium.

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

Seven months following initiation of vitamin D therapy, the value fell to 142.1% of the total body calcium. The results are consistent with the reduction in bone accretion and resorption rates that have previously been noted in patients with hypoparathyroidism.

Findings of negative calcium balance and osteoporosis have been reported for acromegalic patients. A greatly increased bone turnover rate was observed in this disorder, with both accretion and resorption rates elevated. It is not yet clear what mechanism is responsible for these changes. Somatotropin increased bone collagen and protein synthesis; it also acts on kidney and intestine. Hypersomatotropism is generally marked by an increased retention of phosphorus along with an increased urinary calcium excretion. This condition may provoke a response of hyperparathyroidism, which tends to counteract the effects of somatotropism. Some evidence exists to support the hypothesis of hyperparathyroidism super-imposed on hypersomatotropism.

The calcium levels of the hypersomatotropic patients in this study were often greatly elevated. Two male patients, for example, had calcium levels as high as 1600 g, in comparison to the 1150 g characteristic of a normal adult male. In terms of their expected calcium values, the mean calcium of a group of 12 patients was 104.4%. Although there is evidence of hyperphosphatemia in acromegalic patients, the mean P/Ca ration for the patients in this study fell in the normal range (0.55). After radiation therapy was administered to 7 patients, a decrease in the mean calcium to 91.6% of the expected value was noted.

There are conflicting reports of bone involvement reported for diabetes. Insulin affects both bone collagen and total protein synthesis. It was found that for female diabetic patients the total skeletal mass was slightly lower than the range for normals: 93.0% of the total body calcium. Although there were wide variations in the phosphorus level, the mean P/Ca ratio for the group was within the normal range.

These studies show that the TBNAA technique provides useful data for research in that it quantitatively links endocrine function with two parameters of skeletal metabolism: total body calcium and total body phosphorus. The data obtained are useful in elucidating the mechanisms by which endocrines influence skeletal mass and in the evaluation of therapeutic programs.

Since chronic alcoholism has been shown to result in a type of osteoporosis Ca-47 kinetic techniques and in vivo neutron activation techniques were employed to study the mechanism of this osteopenia. The results to date indicate a loss of body calcium in only two of the six alcoholics studied. Total body potassium in these subjects appears normal.

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

The Pu-238, Be neutron irradiation facility currently employed for total-body neutron activation analysis has shown itself to be superior in many respects to the previously employed 14 MeV neutron generator. In the first year of its operation, over 300 activation analyses have been performed on patients. The precision of the technique for measurement of calcium, sodium, and chlorine has been ± 1, 2, and 2% respectively. The precision for the measurement of phosphorus however, has not been satisfactory (± 4%). Efforts are being made to improve the precision of this latter measurement primarily by means of an improved computer program for analysis of the gamma spectral data. Efforts are also in progress to improve the operation of the activation technique and to apply it to the analysis of other elements in the human body. (Cohn)

In the efforts to define the radiation dose response curve between zero and the natural dose rate, screening studies directed to the selection of the most suibable organism and end point were continued. Most of the organisms considered were found to have such small responses at low doses as to not be useable for obtaining statistically significant results with feasible population numbers. The best organism now appears to be a strain of Eschericia coli identified as ATCC strain #10798 C.E. Clifton K-12 (λ). In "Etude Radiobiologigue Du Systems Lysogene D'E.Coli K12. II Induction Par Les Rayons X. Etude Des Faibles Doses. "Annales de L'Institute Pasteur 90, 458-81 (1956), Marcovich reported on the effects of x rays on the induction of K-12(λ) to produce λ phage, using radiation doses as low as 0.3R. This is apparently the lowest dose for which an effect has been recorded in bacteria. In the current studies the K12 (λ) is grown under various conditions, the free λ phage is harvested and the phage is plated with the indicator strain, ATCC # 12435, J. Lederberg W 1485. The end point measured is the number of plaques formed per plate. When grown under normal background conditions in a medium with reduced potassium content there is one plaque per 10^4 cells. The results under reduced background conditions are not available yet. Another aspect of the study is underway using 250 KVP x rays at various doses up to 1200 R to verify the dose-effect curve reported by Marcovich.

Among the new computing programs developed is one that generates theoretical x-ray spectra associated with a given KVP and with specified filters. This is part of a combination of theoretical and experimental approaches to the development of special filters that utilize the K-edge discontinuity in the mass absorption coefficients of various elements to improve radiographs and to reduce patient radiation doses by cutting out x rays of energies above and below those desired. This method may also become useful in enhancing the contrast associated with certain labeling elements, particularly iodine, in problems such as gall bladder visualization and in thyroid studies. Combinations of silver, tin, rhodium, gadolinium, and cadmium filters used with 30, 35, 40, 70, 80, and 90 KVP

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

x-ray spectra were studied. The use of solid state detectors with their superior energy resolution capabilities has improved the experimental characterization of x-ray spectra. This work is in conjuction with research reported in RX-01-03-(c).

The program for analyzing data from the positron detector system has been replaced by one that uses a method quite different from the previous ones, with a great improvement in the output character. The new method depends on the principle that the data can be represented as points on a sampling mesh that corresponds to the zeroes of one of the classical Chebychev polynomials of the second kind, and these polynomials are transforms of a system of orthogonal polynomials that describe the counting distribution. A computationally efficient program based on this was developed and is in use. There have also been changes made in the electronics that eliminate the magnetic drum previously used for data storage and now transmit the signal directly to the Sigma 2 for storage in core memory. These changes have made it possible to begin using the apparatus to study the kinetics of distribution of Krypton-79 in the brain.

A computer program for recording rat breeding information makes it possible to trace the ancestry of a specified animal and to provide weaning and mating summaries. This was developed at the request of the group involved in studying the hereditary aspects of hypertension (RX-01-03-b). The clinical chemistry program has been revised extensively, and now prints out the data on individual patients in a format suitable for inclusion in the hospital charts. Another new program provides a statistical analysis of the correlation of selected clinical parameters with the results of performance tests in parkinsonism patients (RX 01-03-a). The EPR simulation capability has been increased (RX-03-02-c).

Changes in the computer configuration include the addition of a large storage display for the EPR system and a nine track magnetic tape unit. The latter makes it possible to communicate with outside users with industrial standard tapes. At the central facility, the BROOKNET interface has been replaced by BROOKNET II. The Medical Department's Sigma 2 computer was used in some of the critical pre-operational testing of the new system. The necessary changes of the Sigma 2 to handle BROOKNET II communications have been achieved with a corresponding improvement of service. It may be noted that the Medical Department's Sigma 2 is now reported to have the most peripheral attachments of any Sigma 2 installation. These include three magnetic tape drives, a card reader, a paper tape reader, a fast printer, a disc file, a teletype, on-line interfaces to the whole-body counter, and the EPR and another general purpose on-line interface used with the positron detector system and for other occasional applications.

A number of computer programs have been put into FOCUS files. FOCUS is a computer system involving a central Control Data 3200 that is connected

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

to the Control Data 6600's, with user access via data terminals, using telephone lines. It has editing and other file-handling capabilities. The editing feature has proved to be particularly useful in developing new programs such as the one used for computing x-ray spectra. It is also usful in updating and executing programs that involve periodically adding relatively small amounts of data to a larger set of data that continues to be used. For other jobs that would normally be read in from cards the existence of FOCUS files give the convenience of ready availability without card storage and handling. The output from jobs submitted to the 6600 via FOCUS are usually transmitted via BROOKNET to the Sigma 2 for printout.

(Robertson)

17. Expected Results in FY 1974:

The clinical trials with synthetic calcitonin will be continued with 18 patients on a low dose level (0.5 U/Kg/3 times per week). The clinical trials involving human growth hormone administration to osteoporotic patients will be continued for one year. Another group of osteoporotic patients will receive long-term therapy with estrogen, androgens or fluoride singly and in combination. Effective treatment in all these groups of patients should result in an augmentation of their skeletal mass. This will be measured by in vivo neutron activation analyses at intervals during long-term therapy. The effects of physical therapy in mildly osteoporotic women will be evaluated over a one year period.

Studies designed to measure the extent and rate of loss of calcium in patients receiving long-term therapy with the drug colestipol for hyperlipemia will be instituted. The study of osteopenia resulting from endocrine dysfunction, Thalessemia and Paget's disease will continue. The effects of various therapies will be evaluated in terms of changes in total calcium and potassium.

The study of renal osteodystrophy in patients undergoing chronic hemodialysis will be expanded. A number of these patients will be studied using Ca-47 kinetic techniques while they are on dialysis. The effect of various therapies, principally vitamin D analogues will be studied in uremic patients. The study, initiated on patients with renal failure scheduled to undergo renal transplantation, will be expanded.

[redacted] applications for the in vivo activation technique will be sought. For example, changes in body composition resulting from high levels of growth hormone associated with long-term L-Dopa treatment for parkinsonism will be studied. (Cohn)

In the radiation dose response curve studies, an effort will be made to

(See Continuation Sheet)

17. Expected Results in FY 1974: (Cont'd.)

accumulate statistically significant data on the K12 strain grown under control conditions (normal background) and under reduced background radiation conditions. The latter will include the use of K-40 free growth media.

A CRT data terminal will be interfaced with the Sigma 2 to improve the program editing capabilities. A multi input interface and rebuilding of the monitor is planned for better communication with users. It is planned to improve the access to BROOKNET from the EPR keyboard and to provide direct access to BROOKNET from the whole-body counter. (Robertson)

18. Expected Results in FY 1975:

The general objectives will remain the same with modifications based on current findings and development of advanced techniques. The emphasis will be on those clinical applications in which the technique of neutron activation will play the essential role. (Cohn)

Consideration will be given to related experiments with other organisms to study the role of natural background in producing the normal mutation rate. It is expected that Drosophila will probably be the highest order species that can be used because of the problem of having sufficient numbers for statistical significance.

19. Description and Explanation of Major Materials, Equipment and Subcontract Items:

FY 1975 Capital Equipment:

Specific costs for these studies include replacement of two multichannel analyzers one at $10,000, and one at $14,000. Miscellaneous instrumentation equipment, power supplies, amplifiers, recorders, and scalers and two terminal stations for information retrieval and input is estimated at $20,000.

20. Proposed Obligations for Related Construction Projects:

None

(See Continuation Sheet)

Exposure to External and Internal Radiation
In Vivo Measurement of Radionuclides in Man; Body
Project Title: Burden and Kinetic Factors. Computer Applications RX-01-01-(b)

Reiteration of Assurance Statement
on
Investigation Involving Human Subjects
Including Clinical Research

The Hospital of the Medical Research Center, Brookhaven National Laboratory, complies with the policy for the protection of human subjects participating in activities supported directly or indirectly by grants or contracts from the Department of Health, Education, and Welfare and from the Atomic Energy Commission. In fulfillment of its assurance, this institution has established a standing committee to review investigations involving human subjects, including clinical research, and said committee is charged with responsibilities to determine for each proposed study that:

the rights and welfare of each patient will be adequately protected,
that the potential benefits outweigh the risks, and
that an informed consent will be obtained from each patient.

The committee, appointed by the Director, Brookhaven National Laboratory, provides for independent judgment of its members, and alternates are provided to serve when there may be conflict of interest. This institution encourages constructive communication between the committee and principal investigators on the safeguarding of the rights and welfare of subjects; will provide the required care for persons injured as a result of participation; and maintains complete documentation of proposals and reviews. Approved investigations are reviewed by the committee at least annually and the institution periodically reviews its practices and procedures to assure they are consistent with the policies published by the Department of Health, Education, and Welfare.

As of March 7, 1973, the Institutional Review Committee for review of Medical Department research activities involving human subjects is composed of:

J. S. Robertson, M.D., Chairman
G. C. Cotzias, M.D., Alt. Chairman
S. H. Cohn
E. A. Popenoe, Alt. for Dr. Cohn
G. Chikkappa, M.D.
H. Connell
R. A. Love, M.D.
G. Price
N. P. Rathvon, Jr.
D. N. Slatkin, M.D., Alternate
A. P. Wolf, Alternate
L. K. Dahl, M.D. - Clinical Consultant
W. A. Finn - Administrative Consultant
C. W. Flood - Health Physics Consultant

Signature: Eugene P Cronkite

Title: Chairman, Medical Department

Date: March 7, 1973

RX-23

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SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory — RX-Biomedical & Environmental Research
Laboratory — Program

1. Contractor: Associated Universities, Inc.
Contract No.: AT(30-1)-16
Task No.:

2. Project Title:
Exposure to External and Internal Radiation Evaluation of Hazards from Tritium--Dominant Lethal Mutations in Mice
189 No.: RX-24

3. Budget Activity No.: RX-01-01-(c)

4. Date Prepared: May 1973

5. Method of Reporting:
Scientific Meetings
BNL Monthly Letter to AEC
Scientific Journals

6. Working Location:
Brookhaven National Laboratory

7. Person in Charge:
E. P. Cronkite

Principal Investigator:
A. L. Carsten

8. Project Term:
Continuing

From: To:

9. Man-Years:

Direct Man-Years	FY 1973	FY 1974	FY 1975
Scientific & Professional	---	---	---
Other	2.0	2.5	2.5
Guests & Res. Collaborators	---	---	---
Total	2.0	2.5	2.5

10. Costs (In Thousands of Dollars):

	FY 1973	FY 1974	FY 1975
Research Division	70	71	74
Hospital Division	0	0	0
Research Costs	70	71	74
Equipment Obligations	1	1	2

11. Reactor Concept:

12. Materials:

Project Title: Exposure to External and Internal Radiation
Evaluation of Hazards from Tritium--Dominant Lethal
Mutations in Mice RX-01-01-(c)

13. Publications:

Elias, J. M., Conkling, K., and Makar, M. Cold fuelgen hydrolysis; its effect on displacement of tritiated thymidine. Stain Technol. (in press).

16238

14. Scope:

A) 200 Word Summary:

With the increasing number of power reactors, there is a growing concern as to the genetic and somatic effects resulting from exposure to radioactive reactor effluents. Critics contend that the present radiation safety standards, from a genetic and long-term somatic standpoint, are not stringent enough. The objective of these studies is to ascertain if dominant lethal mutations are induced in mice by exposure to 1-100 times the present maximum permissible concentrations (MPC's) of tritium in water.

B) Supplement to 200 Word Summary:

Parent (P_1) animals are fed on tritiated water commencing at four weeks of age. Four weeks later, when their tissues are nearly in equilibrium with HOH-3, they are mated. The offspring (F_1) are maintained on tritiated water throughout the remainder of the experiment. At eight weeks of age they are divided into three experimental groups and mated as follows: group one, consisting of tritiated males mated to tritiated females; group two, consisting of tritiated males mated to untreated females; and group three, consisting of tritiated females mated to untreated males. This allows an estimate of the effect on the male and female germ cells separately and also detection of any interaction if it exists. Initially each experimental group, receives 100 times the MPC. An additional group of untreated controls also is examined.

Histological examination of pre-implantation zygotes is made on a sufficient number of animals to establish that pre-implantation and early post-implantation deaths are due to chromosome abnormalities of the type characteristic for dominant lethal mutations. Serially sectioned ovaries from the F_1 mice are microscopically analyzed to enumerate the number of surviving ova. For comparison, additional groups of x-irradiated animals will be studied.

The concentration of tritium in body water and DNA of cells is measured. The complete tritium analysis includes determination of the amount of activity in the wet tissue, dried residue (freeze dried), DNA, RNA, histones, and residual chromosome associated protein. From these data the dose to ova and testes will be calculated, probable effects estimated, and these compared to observed effects in order to determine if there is any unique and unexpected effect from tritium in germ cells.

(See Continuation Sheet)

Exposure to External and Internal Radiation
Evaluation of Hazards from Tritium--Dominant Lethal
Project Title: Mutations in Mice RX-01-01-(c)

15. Relationship to Other Projects:

This work is closely related to the present studies by the late Dr. Gonzalez of Columbia University and BNL on the genetic effects of radiation in Drosophila melanogaster. At ORNL, W. Russell, L. Russell, and E. Oakburg are currently determining the incidence of recessive mutations from exposure to tritium.

M. Lyon at M.R.C., Radiobiology Research Unit, Harwell, England, and A. C. Bateman, Christie Hospital and Holt Radium Institute, Manchester, England, have been working on the radiation induction of dominant lethal mutations in rodents.

16. Technical Progress in FY 1973:

A total of 1,780 female animals have been bred. These included 748 on tritium and bred with males on tritium, 335 bred to males on regular water, 336 on normal water bred to males on tritiated water, and 361 control animals where both partners were on normal water. Of the 1,780 animals, 1,017 became pregnant and 10,904 corpora lutea were counted. While these figures represent a rather significant number of animals, indications are if a genetic effect does exist, it is not great enough to be readily apparent. Computer programs are currently being written to enable a detailed examination of the data available.

Animals were examined for incorporation of tritium for as long as 302 days after initiation of the experiment. Preliminary results indicate that by 14 days after beginning on tritium water, the plasma and wet tissue tritium activity has reached more than 80% of the values measured at 302 days. The dry tissue activity similarly plateaus within the first 30 days on tritiated H_2O.

After maintenance on tritium for 37 weeks, animals have been sacrificed, the bone marrow harvested from the hind legs, and evaluated for stem cell content as measured by the spleen colony technique. Initial results indicated a reduction in stem cell number in the tritiated animals.

A pilot determination is now in progress aimed at measuring the amount of tritium incorporated into DNA, RNA, histone and residual chromosome associated with protein. As yet this work is too preliminary for comment, however, the techniques were standardized.

17. Expected Results in FY 1974:

Accumulation of information on the long-term incorporation of tritiated water into the various body compartments and nuclear constituents will continue in order to determine whether or not maintenance on 100 x the MPC will

(See Continuation Sheet)

Exposure to External and Internal Radiation
Evaluation of Hazards from Tritium--Dominant Lethal
Project Title: Mutations in Mice RX-01-01-(c)

17. Expected Results in FY 1974: (Cont'd.)

cause genetic damage as measured by the dominant lethal test. Additional evaluations of the hematopoietic system will be made at various times to determine whether there is an effect on the bone marrow. Due to budget limitations, the histological examinations of the embryos which was indicated as a part of this program in last year's budget report have not been undertaken. It is hoped that work on this phase of the project might be started.

18. Expected Results in FY 1975:

This program will continue and data will be accumulated on the effectiveness of both tritiated water and X-ray exposures in producing dominant lethal mutations. Studies evaluating the effects of both treatments on the hematopoietic stem cell pool should also be completed.

19. Description and Explanation of Major Materials, Equipment and Subcontract Items:

None

20. Proposed Obligations for Related Construction Projects:

None

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory — RX-Biomedical & Environmental Researc
Laboratory — Program

1. Contractor: Associated Universities, Inc. — Contract No.: AT(30-1)-16 — Task No.:

2. Project Title: Combating Detrimental Effects of Radiation
The Role of Stem Cell and Therapy of the Hemorrhagic Phase in Radiation Injury — 189 No.: RX-28

3. Budget Activity No.: RX-01-02

4. Date Prepared: May 1973

5. Method of Reporting:
Scientific Meetings
BNL Monthly Letter to AEC
Scientific Journals

6. Working Location:
Brookhaven National Laboratory

7. Person in Charge:
E. P. Cronkite
V. P. Bond
Principal Investigator:
A. L. Carsten
A. D. Chanana
H. Burlington, Mt. Sinai University, N. Y.

8. Project Term:
This effort reported in RX-03-02-(b) in FY 1974 and FY 1975

9. Man-Years:	FY 1973	FY 1974	FY 1975
Sci., Res. Assoc. (Ph.D. or Equiv.)	1.0		
Visiting Sci.	---		
Prof. (B.S. or Equiv.)	---		
Sci. & Prof. - Total	1.0	---	---
Technical	2.5	---	---
Adm. & Clerical	---	---	---
Guests & Research Collaborators	---	---	---
Total	3.5	---	---

10. Costs (In Thousands of Dollars):	FY 1973	FY 1974	FY 1975
Labor (including benefits)	65		
Mats., Trav., Dev. Subcont., Spec'l Proc.	8		
Reactor, Accel., and/or Computer Usage	0		
Allocated Technical Services	0		
Gen. & Adm. Overhead			
Total Research Cost		0	0
Research Division	103		
Hospital Division	0		
Research Costs	103	0	0
Equipment Obligations	2	0	0

11. Reactor Concept:

12. Materials:

Project Title: Combating Detrimental Effects of Radiation
The Role of Stem Cell and Therapy of the Hemorrhagic Phase in Radiation Injury RX-01-02

13. Publications:

Matsui, K., Carsten, A. L., and Cronkite, E.P. Effects of storage on CFU of mouse bone marrow cells. J. Radiat. Res. 13, 59-70 (1972). 14612

Burlington, H., Cronkite, E. P., Reincke, U., and Zanjani, E. D. Erythropoietin production in cultures of goat renal glomeruli. Proc. Nat. Acad. Sci. 69, No. 12, 3547-50 (1972). 17096

Cronkite, E. P., and Fliedner, T. M. The radiation syndromes. Handbuch der Medizinischen Radiologie, Fritz-Niggli, Editor, Bergmann Verlag, Munchen (in press). 17319

14. Scope:

A) 200 Word Summary:

An effective therapy of the hemopoietic phase of whole body radiation injury requires histocompatible stem cells for transplantation. Thus, the primary objective in this budget activity is to gain more basic knowledge of the properties of and the relationship between pluripotent cells and committed stem cells. Marrow aplasia is a major cause of the deaths that occur following accidental exposure to whole body radiation and during immunosuppression and chemotherapy of malignant disease. If a practical method for transplanting bone marrow were developed, more extensive chemotherapy for immunosuppression and treatment of radiation exposure accidents as well as malignant disease would be possible. At present there are no practical methods for stockpiling histocompatible lymphocytic antigen (HL-A) characterized bone marrow for use in man, although substantial progress has been made in the development of techniques for preservation of mouse and canine bone marrow.

Because of the close relationship of these studies to those reported in RX-03-02-(b) this work will be reported in RX-03-02-(b) in subsequent years.

15. Relationship to Other Projects:

Related studies by Lewis and Trobaugh at St. Luke's Hospital, Chicago, and by Wolf and Trentin at Baylor University, are concerned with the factors determining the type of spleen colony arising from marrow injections into irradiated recipients. However, they are not examining the same factors as at B[redacted]or are they examining the total spectrum of colony type and size over the e[redacted]re development period for both endogenous and exogenous colonies as in this study.

All spleen colony work is, of course, related to the work of Till and McCulloch, University of Toronto, since they developed the technique and continue to use it in a variety of studies. Sensenbrenner and Santos, Johns

(See Continuation Sheet)

Project Title: Combating Detrimental Effects of Radiation
The Role of Stem Cell and Therapy of the Hemorrhagic Phase in Radiation Injury RX-01-02

15. Relationship to Other Projects: (Cont'd.)

Hopkins Medical Center, are interested in the effects of drugs on stem cell growth, with essentially no work related to radiation effects. The studies involving cyclophosphamide effects are closely related to studies here: Sensenbrenner collaborates with BNL on the diffusion chamber technique. H. Ragan, Battelle Northwest, who learned the diffusion chamber technique at BNL, is interested in the effects of internal emitters on the hematopoietic system.

Stohlman's group at St. Elizabeth's Hospital, Brighton, Massachusetts, is interested in all aspects of normal and abnormal hematopoiesis. Benestad of the Norwegian Defense Research Establishment was a key figure in the development of the implanted diffusion chamber and the application of his method in collaboration with his colleague, A. Boyum, a former Research Collaborator in Residence at BNL.

16. Technical Progress in FY 1973:

Attempts to produce large numbers of HSC in diffusion chamber cultures have to date been unsuccessful. Attempts will continue in view of the potential clinical application.

During this year the diffusion chamber culture technique has been applied to the study of bone marrow from patients with various blood dyscrasias. Nine patients were studied (chronic myeloid leukemia 4, acute myelomonocytic leukemia 3, myelofibrosis with myeloid metaplasia 2, polycythemia vera 1). These observations were compared with the growth patterns of 3 normal bone marrow samples. In normal bone marrow an orderly maturation of cells took place similar to that seen in vivo. Autoradiographic studies on marrow from the patients with acute myelocytic and chronic myelocytic leukemia suggested a faster maturation rate than that previously reported. The rate of maturation in the diffusion chamber may be somewhat slower in myelofibrosis and chronic myeloid leukemia (CML) as compared with the normal individual. When bone marrow from patients with Ph_1 + CML and leukocyte alkaline phosphatase (LAP) negative cells is grown in diffusion chambers the Ph_1 + mitoses persist but a progeny of intensely positive LAP cells emerges. This proves that LAP negativity is not due to loss of LAP genomes when deletion of autosome 23 produced the Ph + chromosome. The LAP negativity is due to repression of the genomes carrying the code for LAP in CML. Their derepression by factors supplied by the marrow environment results in LAP + cells.

The RBE of the CFU has been studied using the spleen colony technique in mice exposed to various energy of neutrons. The D_0 values obtained were as follows: 250 KVp x rays - 80.14 rads, reactor fission neutrons-40.78 rads, accelerator neutrons - 0.4 MeV - 28.14 rads; 0.66 MeV neutrons - 34.50 rads; 1.0 MeV neutrons - 28.12 rads; 1.5 MeV neutrons - 28.8 rads; 1.8 MeV neutrons - 29.62 rads.

(See Continuation Sheet)

Project Title: Combating Detrimental Effects of Radiation
The Role of Stem Cell and Therapy of the Hemorrhagic Phase in Radiation Injury RX-01-02

17. Expected Results in FY 1974:

See RX-03-02-(b)

18. Expected Results in FY 1975:

See RX-03-02-(b)

19. Description and Explanation of Major Materials, Equipment and Subcontract Items:

See RX-03-02-(b)

20. Proposed Obligations for Related Construction Projects:

See RX-03-02-(b)

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory — Laboratory

RX-Biomedical & Environmental Research — Program

1. Contractor: Associated Universities, Inc.
Contract No.: AT(30-1)-16
Task No.:

2. Project Title: Nuclear Medicine Technology and other Health Applications Treatment and Biochemical Dissection of Parkinsonism and Allied Conditions
189 No.: RX-34

3. Budget Activity No.: RX-01-03-(a)

4. Date Prepared: May 1973

5. Method of Reporting:
Scientific Meetings
BNL Monthly Letter to AEC
Scientific Journals

6. Working Location: Brookhaven National Laboratory

7. Person in Charge: G. C. Cotzias

Principal Investigator: G. C. Cotzias

8. Project Term: Continuing
From: To:

9. Man-Years:

Direct Man-Years	FY 1973	FY 1974	FY 1975
Scientific & Professional	3.5	3.5	3.5
Other	31.0	31.5	34.5
Guests & Res. Collaborators	8.5	6.0	6.0
Total	43.0	41.0	44.0

10. Costs (In Thousands of Dollars):

	FY 1973	FY 1974	FY 1975
Research Division	302	323	342
Hospital Division	490	554	615
Research Costs	792	877	957
Equipment Obligations	7	13	23

11. Reactor Concept:

12. Materials:

Project Title: Nuclear Medicine Technology and other Health Applications
Treatment and Biochemical Dissection of Parkinsonism and Allied Conditions RX-01-03-(a)

13. Publications:

Cotzias, G. C., Tang, L. C., Miller, S. T., Sladic-Simic, D., and Hurley, L. S. A mutation influencing the transport of manganese, levodopa and tryptophan. Sci. 176, 410-12 (1972). 16300

Mena, I., Lopez, G., Horiuchi, K., and Croxatto, H., Jr. Low viability of newborns of levodopa treated rats. Nature, 239, 285-7 (1972). 16303

Cotzias, G. C., Lawrence, W. H., Papavasiliou, P. S., Düby, S. E., and Mena. I. Apomorphine and parkinsonism. Trans. Am. Neurol. Assoc. 97, 156-9 (1972). 16490

Papavasiliou, P. S., Cotzias, G. C., Düby, S. E., Steck, A. J., Bell, M., and Lawrence, W. H. Melatonin and parkinsonism. J. Am. Med. Assoc. 221, No.1, 88 (1972). 16698

Cotzias, G. C., Tang, L. C., and Mena, I. Effects of inhibitors and stimulators of protein synthesis on the cerebral actions of levodopa. Neurosciences Research, I. J. Kopin and S. Ehrenpreis, Editors, Vol. 6, Academic Press, New York, 1972 (in press). 16732

Düby, S. E. Cotzias, G. C., Papavasiliou, P. S., and Lawrence, W. H. Injected apomorphine and oral levodopa in parkinsonism. Arch. Neurol. 27, 474-80 (1972). 16776

Cotzias, G. C., Lawrence, W. H., Papavasiliou, P. S., and Düby, S. E. Nicotinamide and parkinsonism. New Engl. J. Med. 287, 147 (1972). 17003

Ginos, J. Z., LoMonte, A., Cotzias, G. C., Bose, A. K., and Brambilla, R. J. Synthesis of tritium- and deuterium- labeled apomorphine. J. Am. Chem. Soc. (in press). 17155

Cotzias, G. C. Controlled double-blind studies of drugs. New Engl. J. Med. 287, 937 (1972). 17183

Cotzias, G. C. Metabolic responses to levodopa. New Engl. J. Med. 287, 1302-3 (1972). 17295

Cotzias, G. C., Papavasiliou, P. S., and Mena, I. L-meta-tyrosine and parkinsonism. J. Am. Med. Assoc. 223, No. 1, 83 (1973). 17304

Cotzias, G. C. Levodopa and related drugs. The Medical Letter (in press). 17307

Gillespie, N. G., Mena, I. Cotzias, G. C., and Bell, M. A. Diets for modifying responses to levodopa in parkinsonism. J. Am. Dietet. Assoc. (in press). 17328

(See Continuation Sheet)

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13. Publications: (Cont'd.)

Papavasiliou, P. S., and Cotzias, G. C. Levodopa and dopamine in cerebrospinal fluid. Neurology (in press). 17342

Cotzias, G. C., Mena, I., and Papavasiliou, P. S. Overview of present treatment of parkinsonism with levodopa. Presented at the Symposium on Dopa Decarboxylase Inhibitors--Their Role in the Treatment of Parkinsonism, New York City, New York, November, 1972. 17444

Mena, I., Cotzias, G. C., Brown, F. C., Papavasiliou, P. S., and Miller, S. T. Defective release of growth hormone in parkinsonism improved by levodopa. New Engl. J. Med. 288, No. 6, 320-1 (1973). 17509

Cotzias, G. C. Levodopa, manganese and degenerations of the brain. The Harvey Lectures, Acedemic Press, New York (in press). 17511

Papavasiliou, P. S., Cotzias, G. C., Mena, I., and Bell, M. Sodium gamma-hydroxybutyrate for parkinsonism. J. Am. Med. Assoc. (in press). 17573

14. Scope:

A) 200 Word Summary:

The purpose of the current work is to comprehend better the mechanisms by which levodopa exercises its primary effects and side effects in patients with parkinsonism, and thus to develop even more effective management. The "off-on" phenomenon, a late defect in the chronic treatment with levodopa, was controlled in susceptible patients either by: 1) co-administering with levodopa the peripherally acting dopa-decarboxylase inhibitor, α-methyldopa hydrazine (MK-486); or these drugs combined, plus a moderate restriction of dietary protein intake. This latter regimen induced symptomatic stability as well as higher levels of circulating growth hormone. The possible role of this hormone in the treatment of parkinsonism is therefore studied further.

The drug apomorphine, which contains a piperidine-like moiety in addition to a dopamine-like moiety in its structure, improved parkinsonism without inducing the mental aberrations expected from treatment with levodopa in susceptible patients. Oral administration of apomorphine, however, had induced a dose-dependent azotemia. Therefore, N-n-propylnoraporphine is being tried, which is 50 times more potent than apomorphine and might therefore require 1/50th of the dose of apomorphine for oral therapy. The presence of the piperidine moiety in apomorphine suggested that piperidine should be tried separately against mental and motor side effects of levodopa. Trials of piperidine are therefore being prepared.

(See Continuation Sheet)

Project Title: Nuclear Medicine Technology and other Health Applications
Treatment and Biochemical Dissection of Parkinsonism and Allied Conditions RX-01-03-(a)

14. Scope: (Cont'd.)

B) Supplement to 200 Word Summary:

The objectives remain the study and correction of biochemical defects of extrapyramidal diseases which were treated first with levodopa alone and later with a combination of levodopa and the decarboxylase inhibitor α-methyldopa hydrazine (MK-486). Definition of the precise mechanism of the short-lived episodic dimunition of the treatment's effectiveness and of the involuntary movements and mental side-effects is sought.

Since dopamine, the active metabolite of dopa, administered systemically does not cross the blood-brain barrier in sufficient amounts to elicit cerebral effects, apomorphine, a dopaminergic tertiary amine, is used in order to study dopaminergic effects on the central nervous system. The therapeutic effects of apomorphine are similar to those of levodopa whereas most side-effects are less. Oral apomorphine, however, induced an apparently dose-dependent azotemia; its analogue, N-n-propylnoraporphine, which requires 1/50th the dose of apomorphine, is now studied.

Dietary and hormonal factors influence the effects of levodopa. There is particular interest in exploring the role of growth hormone (GH) in man; also the effect of levodopa is potentiated by growth hormone in animals. Since this hormone is investigated as a treatment of osteoporosis, the total-body neutron activation analysis technique described in RX-01-01-(b) is used to study changes in total-body calcium of parkinsonians receiving levodopa. In animals, the use of monoamine oxidase inhibitors to prevent the destruction of dopamine demonstrated that its cerebral effects are similar to those of levodopa. One might be able therefore to utilize metabolic inhibitors to promote the entrace of C-11-dopamine into the brain and thus develop a method for scanning intracerebral structures.

The discoveries of various interactions between dopamine and manganese led to development of a nondestructive analysis for manganese permitting subsequent analysis for dopamine in the same biological sample. Application of this method to regional samples of cat brains is in progress. Furthermore, manganese radioisotopes have permitted the evaluation of the cerebral reactions of animals to levodopa and thus Mn-54, used as a marker may enable prediction of cerebral reactivity to drugs such as levodopa.

15. Relationship to Other Projects:

The extensive investigations stimulated by the introduction of levodopa for the treatment of parkinsonism have been extended toother extrapyramidal disorders and have stimulated a search for newer and better drugs by numerous other centers. The neurochemical studies that evolved have branched out more recently to studies of hormonal interactions as well as pituitary-tropic inhibiting factors as modifiers of the effects of dopaminergic drugs.

(See Continuation Sheet)

15. Relationship to Other Projects: (Cont'd.)

Parkinsonism is being studied intensely by Dr. A. Barbeau in Montreal; by Dr. M. Yahr at Columbia; by Dr. F. McDowell at Cornell; by Dr. A. Battista at N.Y.U.; and many other leaders of groups elsewhere. Neurochemical work is being performed in so many excellent centers that it is impossible to choose even representative examples.

Related studies at BNL include those using total body neutron activation analysis as reported in RX-01-01-(b) and utilization of C-11-dopamine as reported in RX-01-03-(c). Dr. Kraner of the BNL Instrumentation Division contributes essentially to the development of instrumentation in this project.

16. Technical Progress in FY 1973:

α-methyldopa hydrazine (MK-486) in combination with levodopa emerges as a most useful therapeutic tool as a consequence of inhibiting the catabolism of levodopa exclusively in peripheral tissues. It has lowered the dose requirements of levodopa by approximately 80%, while it has induced more rapid therapy, better diurnal symptomatic control, and a new capability to co-administer pyridoxine with impunity in patients receiving levodopa. Its main disadvantage is the rapid induction of involuntary movements. Fixed combinations of levodopa with MK-486 and a multiple dose schedule were proposed for the successful application by practitioners of this new form of therapy for parkinsonism.

Despite previous publications to the contrary, melatonin did not affect the signs of parkinsonism, the therapeutic effects of levodopa, or adventitious movements. A tranquilizing effect was encountered, therefore suggesting that melatonin be tried for manic-depressive disorders. Although animal experiments with nicotinamide were found encouraging, clinical trials proved the vitamin to be ineffective in controlling dopa-induced involuntary movements in patients. Similarly, although many theoretical considerations and animal experiments suggested that L-meta-tyrosine would show improvement of parkinsonism there was no demonstrable improvement observed. It was concluded from these experiments, however, that the catechol or an equivalent configuration is necessary, since this is the only difference between L-meta-tyrosine and L-dopa.

Injected apomorphine was found to be effective against tremor, rigidity, and the episodic bradykinesia when given both alone and with oral levodopa. Yet, the dopa induced "awakening effect", the dyskinesia and the nausea were often antagonized by apomorphine whereas the sedative effects and nausea of apomorphine were antagonized by levodopa. The coexistence of both synergistic and antagonistic effects between the two drugs may be a result of the structure of apomorphine, a hybrid drug by virtue of containing several neuro-active moieties. Slow administration of oral apomorphine to 14 patients up to dosages of 1.5 g/d induced more of the expected side effects, while significant improvement of the parkinsonism was induced in five patients. Unexpectedly, three patients exhibited a reversible marked elevation of

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

both blood urea nitrogen and blood creatinine levels. This suggests such trials be conducted with N-n-propylnoraporphine.

In studies on synthesis of tritium- and deuterium-labeled apomorphine, tritiated apomorphine was synthesized from morphine and from apomorphine by labeling the two aromatic rings and will be used in future animal experimentation. The reported work showed how exclusive labeling of the catechol ring can be achieved by controlling the reaction conditions.

In response to work of others heralding iatrogenic acromegaly in patients receiving levodopa it was found that: 1) spontaneous releases of growth hormone, similar to those evoked by levodopa were found normally during sleep, 2) none of 105 patients with parkinsonism or chronic manganese poisoning treated here have developed acromegaly, 3) large pulses of circulating growth hormone were found only after the morning dose of levodopa, 4) life-long consumption of levodopa has prolonged the lives of mice. In preliminary observations of untreated patients with parkinsonism there was a virtual absence of the circadian rises of growth hormone in contrast to normals in whom several large rises of hormones were spontaneously apparent. The relative inability of the parkinsonian patients to raise growth hormone levels beyond the basal ones seems to be corrected by levodopa. This study is continuing with a larger number of patients and growth hormone levels will be correlated with patient performance.

In studies of the effects of protein metabolism in parkinsonism it was shown, in mice, that agents which inhibit protein synthesis diminish the cerebral effects of levodopa. This result is complementary to the observation that drugs reputed to increase protein synthesis increase the duration and intensity of these effects. Inhibitors of protein synthesis also diminished the effects of apomorphine and oxotremorine whereas inactive analogues did not. The findings suggest that proteins critical to the function of dopaminergic and cholinergic receptors undergo rapid turnover. Some patients reported episodic losses of the therapeutic effects of levodopa following a high protein meal. Since levodopa, a large neutral amino acid, might compete with dietary amino acids for transport into the brain, isocaloric diets were administered with varying protein content and the effects on symptomatic stability determined. These studies suggest the following: 1) neurological effects became evident in most patients with variation of the diet but were most striking when levodopa was administered alone; 2) high protein intake tended to cancel both the therapeutic effects and the side effects of levodopa, 3) low protein diets tended to potentiate and stabilize the therapeutic effects, 4) the fewer the meals, the greater the instability. These results indicate that long-term studies of low protein intakes are in order.

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

These studies (and others) were facilitated by the development of an automated ergometer, for the fast, simple, and quantitative assessment of changes of the neurological state in parkinsonism. The changes in output of work correlated with the neurological changes determined with a conventional scoring system.

Studies on cerebrospinal fluid (CSF) showed levodopa and dopamine to be present only after levodopa administration and not in control patients; however, their levels did not correlate with dose, improvement or side-effects. When L-dopa was given with pyridoxine, the level of levodopa in the CSF dropped and correlated with a loss of therapeutic efficacy. However, a more convenient biochemical marker for the effect of pyridoxine is the determination of urinary dopamine. Thus, the usefulness of CSF analyses in following the treatment of parkinsonism is questioned. Animal experiments are proposed to develop methods applicable to brain biopsies.

In order to maximize the chemical information one can obtain from these biopsies a method was developed to analyze biopsy samples of brain for manganese and catecholamines. This micro-method combines nondestructive neutron activation analysis for manganese with spectrofluoremetric determination of dopa and dopamine in the same sample. Reaction of cats given 200 and 400 mg/kg (i.p.) of saline or levodopa were scored. The animals were then anesthetized for sampling the caudate, the hypothalamus, the corpus callosum, the frontal white and frontal gray cortex. Administration of levodopa was found to increase the concentration of dopa and dopamine in all areas studied. In the caudate area the level of dopamine showed a high correlation with behavioral scores. Ranking of control animals according to dopamine concentration in the caudate had a coefficient of 0.8 with their ranking by manganese concentration in this nucleus. Manganese levels in the hypothalamus appeared to correlate with those of dopamine. Upon completion of this phase of the study regional brain samples from living cats will be obtained stereotactically in preparation for studies in appropriate human subjects.

In other studies on interactions between levodopa and manganese, young mice (three weeks of age) showed both higher responses to levodopa and faster turnover of manganese than adult animals, implying correlations between the two. Data obtained on newborn mice, their mothers, and mice of various ages, include: 1) concentration of Mn in embryonic liver is about 1/6 that of the adult and remains at that level until the 12th postpartum day; 2) at day 18 liver Mn peaks to 11.8 $\pm$ 2.5 μg/g dry weight (adult 3.0 μg/g) in offspring from mothers fed purina chow, in contrast to offspring from mothers fed milk; 3) the peak persisted between days 17 and 19, followed by a slow decline to adult levels at about 5 weeks; 4) Mn-54 was not eliminated from the whole body between birth and the 18th day of life; 5) one week old mice injected with radiomanganese incorporated 0.3% of the

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

tracer into brain after 4 hours and 20% after 30 days; 6) the animals' reactivity to levodopa tended to follow the manganese concentrations. These findings show that young animals are exceedingly susceptible to Mn poisoning early in life since they accumulate excesses of metal spontaneously; have almost no blood-brain barrier against it; and their homeostatic mechanisms are insufficient to deal with these excesses.

Pharmacological parkinsonism induced in psychotic patients by reserpine became a biochemical model in treating idiopathic parkinsonism. Conversely, the induction of pharmacological psychoses while treating parkinsonism with levodopa might constitute biochemical models for spontaneously occurring mental aberrations. Since apomorphine (which can stimulate both dopaminergic and cholinergic receptors) had not induced such psychoses, it appeared desirable to determine whether a cholinergic agent given together with levodopa could prevent psychoses without jeopardizing the control of parkinsonism.

Physostigmine was the first cholinergic agent to be used for this purpose, since it has aggravated parkinsonism only when given without levodopa. A preliminary study of four parkinsonians with levodopa-induced mental aberrations showed that injected physostigmine (0.5 - 1.75 mg s.c.) diminished garrulity, hallucinations and delusions. Adverse effects on parkinsonism were limited to bursts of tremor and occasionally salivation, both of which were mild. On the basis of these observations the FDA has been petitioned for permission to use physostigmine orally on a chronic basis.

Acting as an acetylcholinesterase inhibitor, physostigmine is an indirect stimulator of cholinergic receptors as opposed to oxotremorine, a potent direct stimulator of muscarinic receptors. The oral LD_{50} of oxotremorine (2.59 μg/g in Swiss albino mice) was increased fivefold by pretreatment with 1 - 1.5 μg/day oxotremorine (once a day for 4 days) and sixfold by pretreatment with probanthine. Decreased lethality was also achieved by adding oxotremorine to drinking water (2.5 or 12.5 μg/ml) for one week prior to testing the LD_{50}. The cerebral signs generated from the test injections were similar in both pre-and non-treated animals, suggesting that one can again diminish toxicity without diminishing cerebral effects, as with D,L-dopa, levodopa, and apomorphine.

[redacted]n oxotremorine was given i.p. (0.1 or 0.2 μg/g) into unilaterally caud[redacted]mized mice it caused marked turning opposite from the lesion and runn[redacted] in that direction for several hours. Pretreatment with levodopa (0.3 and 0.4 mg/g i.p.) blocked these effects. When both drugs were given, the animals became immobile but showed no postural changes. These results indicated that one can control the activities of both the dopaminergic and cholinergic systems.

(See Continuation Sheet)

17. Expected Results in FY 1974:

Continuation of studies on cholinergic agents in mice is planned in order to prepare for human investigations.

The studies on manganese metabolism will be continued on animals and extended in man by using Mn isotopes (Mn-54) which it is hoped will serve as a possible indicator of cerebral sensitivity to neuractive drugs. The possibility that injected dopamine may be caused to enter the brain by inhibiting its deamination encourages the use of C-11 labeled dopamine as an agent which may allow visualization of intracerebral structures. If successful in imaging brains of large animals this approach will be extended to humans.

As indicated above oral apomorphine proved to be an effective dopaminergic agent for the control of parkinsonism. With the exception of reversible azotemia induced by high doses of apomorphine it appears safe and induces no dyskinesia or mental aberration. A newly synthesized analogue, N-n-propyl-noraporphine is reported to be about 50 times more effective as a dopaminergic agent than apomorphine. This agent will be given to parkinsonian patients in therapeutic trials anticipating beneficial effects on the extrapyramidal system and no induction of azotemia.

Analogues of apomorphine are being synthesized by combining a catechol moiety with that of a piperidine. Such analogues are potentially dopaminergic with activity increased by alkyl substituents on the piperidine. Spectro-fluorimetric and radioassays are being developed for tracing the metabolic pathways of these analogues.

The work on pharmacological psychoses will be extended from the physostigmine experiments to other direct neuronal cholinergic stimulators not only in parkinsonism but also in investigations of potential treatments of other pharmacological mental aberrations. Proposed agents include piperidine and analogues and oxotremorine.

In collaboration with Dr. S. H. Cohn the endogenous release of growth hormone by levodopa administration will be pursued as a potential treatment of osteoporosis.

18. Expected Results in FY 1975:

Studies will continue with emphasis determined by findings that develop as the work progresses.

(See Continuation Sheet)

Project Title: Nuclear Medicine Technology and other Health Applications
Treatment and Biochemical Dissection of Parkinsonism and Allied Conditions RX-01-03-(a)

19. Description and Explanation of Major Materials, Equipment and Subcontract Items:

FY 1975 Capital Equipment:

A refrigerated, zonal ultracentrifuge with accessory heads is required for processing the large number of samples obtained in animal studies preliminary to clinical trials ($13,000).

20. Proposed Obligations for Related Construction Projects:

None

(See Continuation Sheet)

Project Title: Nuclear Medicine Technology and Other Health Applications
Treatment and Biochemical Dissection of Parkinsonism and Allied Conditions RX-01-03-(a)

Reiteration of Assurance Statement
on
Investigation Involving Human Subjects
Including Clinical Research

The Hospital of the Medical Research Center, Brookhaven National Laboratory, complies with the policy for the protection of human subjects participating in activities supported directly or indirectly by grants or contracts from the Department of Health, Education, and Welfare and from the Atomic Energy Commission. In fulfillment of its assurance, this institution has established a standing committee to review investigations involving human subjects, including clinical research, and said committee is charged with responsibilities to determine for each proposed study that:

the rights and welfare of each patient will be adequately protected,
that the potential benefits outweigh the risks, and
that an informed consent will be obtained from each patient.

The committee, appointed by the Director, Brookhaven National Laboratory, provides for independent judgment of its members, and alternates are provided to serve when there may be conflict of interest. This institution encourages constructive communication between the committee and principal investigators on the safeguarding of the rights and welfare of subjects; will provide the required care for persons injured as a result of participation; and maintains complete documentation of proposals and reviews. Approved investigations are reviewed by the committee at least annually and the institution periodically reviews its practices and procedures to assure they are consistent with the policies published by the Department of Health, Education, and Welfare.

As of March 7, 1973, the Institutional Review Committee for review of Medical Department research activities involving human subjects is composed of:

J. S. Robertson, M.D., Chairman
G. C. Cotzias, M.D., Alt. Chairman
S. H. Cohn
E. A. Popenoe, Alt. for Dr. Cohn
G. Chikkappa, M.D.
H. Connell
R. A. Love, M.D.
G. Price
N. P. Rathvon, Jr.
D. N. Slatkin, M.D., Alternate
A. P. Wolf, Alternate
L. K. Dahl, M.D. - Clinical Consultant
W. A. Finn - Administrative Consultant
C. W. Flood - Health Physics Consultant

Signature: Eugene P Cronkite

Title: Chairman, Medical Department

Date: March 7, 1973

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory — Laboratory

RX-Biomedical & Environmental Research — Program

1. Contractor: Associated Universities, Inc.
Contract No.: AT(30-1)-16
Task No.:

2. Project Title:
Nuclear Medicine Technology and Other Health Applications
Interrelationship between Genetic and Environmental Factors in Clinical and Experimental Hypertension
189 No.: RX-45

3. Budget Activity No.: RX-01-03-(b)

4. Date Prepared: May 1973

5. Method of Reporting:
Scientific Meetings
BNL Monthly Letter to AEC
Scientific Journals

6. Working Location: Brookhaven National Laboratory

7. Person in Charge: L. K. Dahl

Principal Investigator: L. K. Dahl

8. Project Term: Continuing
From: To:

9. Man-Years:

Direct Man-Years	FY 1973	FY 1974	FY 1975
Scientific & Professional	2.0	2.0	2.0
Other	19.0	19.0	21.0
Guests & Res. Collaborators	4.0	1.0	1.0
Total	25.0	22.0	24.0

10. Costs (In Thousands of Dollars):

	FY 1973	FY 1974	FY 1975
Research Division	254	294	303
Hospital Division	235	214	267
Research Costs	489	508	570
Equipment Obligations	3	8	10

11. Reactor Concept:

12. Materials:

Project Title: Nuclear Medicine Technology and Other Health Applications
Interrelationship between Genetic and Environmental Factors in Clinical and Experimental Hypertension RX-01-03-(b)

13. Publications:

Dahl, L. K., Heine, M., and Thompson, K. Genetic influence of renal homografts on the blood pressure of rats from different strains. Proc. Soc. Exp. Biol. Med. 140, 852-56 (1972). 16086

Dahl, L. K., Leitl, G., and Heine, M. Influence of dietary potassium and sodium/potassium molar ratios on the development of salt hypertension. J. Exp. Med. 136, No. 2, 318-30 (1972). 16703

Rapp, J. P. and Dahl, L. K. Suppression of aldosterone in salt susceptible and salt resistant rats. Endocrinology (in press). 17095

Rapp, J. P. and Dahl, L. K. Corticosteroid pattern in rats genetically susceptible to hypertension. Excerpta Med. (in press). 16951

Iwai, J., Dahl, L. K., and Knudsen, K. D. Genetic influence on the renin-angiotensin system. II. Low renin activities in hypertension-prone rats. Circulation Res. (in press). 17322

Rapp, J. P., Knudsen, K. D., Iwai, J., and Dahl, L. K. Genetic control of blood pressure and corticosteroid production in rats. Circulation Res. (in press). 17323

14. Scope:

A) 200 Word Summary:

The etiology and pathogenesis of hypertension (HT) is investigated with emphasis on the interaction between genetic determinants and modifying environmental factors. Clinical and experimental programs originated with the observations that salt (NaCl) intake could modify blood pressure (BP) in man and rat. In man, conclusions had to be based on statistical evaluation of clinical and epidemiological data from groups. In rats, selective breeding produced two colonies with opposite and predictable BP responses of individuals to the same salt intake. These two strains were equally sensitive or resistant respectively, to other hypertensinogenic stimuli so the genetic substratum operates in all "forms" of HT.

Biochemical mechanisms controlled by the multiple genes that modify BP are studied and one that controls a hypertensinogenic mineralocorticoid, 18-hydroxy-deoxycorticosterone, was identified. Since other genes clearly involve the kidney, studies include the effect on BP and renin activity of transplanting between strains: a) whole kidneys, b) parts of kidney, and c) adrenal cortex. Other studies are related to the association of menopause and HT in humans; to a humoral factor that might lead to a clinical test for predisposition to HT; and to a variety of genetically determined functions.

Clinically, the association of HT with gout, diabetes, obesity, and atherosclerosis is studied; particularly interrelations involving lipid,

(See Continuation Sheet)

14. Scope: (Cont'd.)

purine, and carbohydrate metabolism.

B) Supplement to 200 Word Summary:

The unique tool in the experimental studies of this program is the two colonies of rats produced through selective breeding. One colony--R rats--does not develop hypertension except as a consequence of extraordinary insults. The other colony--S rats--develops hypertension readily from a number of non-genetic (environmental) conditions usually considered to be causes of HT.

Blood pressure is regulated by many factors. When any one of these factors varies outside its allotted range, hypertension may develop. A genetic component which determines how much variation is tolerated renders some animals more sensitive then others. Salt intake is one of many triggering factors, and animals sensitive or resistant to salt have proved also to be sensitive or resistant, respectively, to other hypertensinogenic stimuli. Different forms of hypertension, therefore, have more in common than the variety of so-called causative agents might suggest. In applying this reasoning to human hypertension it is suggested that hypertension can result when one or more non-genetic factors interact with the appropriate genetic substrate. Thus, in the individual with high genetic predilection, minimal environmental injury (e.g., salt, kidney disease) might precipitate severe hypertension. At the other extreme, the individual with low genetic predilection might be spared hypertension even after intense and prolonged exposure to the same injurious factors. Given the usual genetic heterogeneity of man, it is likely that the more prevalent individual would be one with middling predisposition who will or will not develop hypertension depending upon such things as the intensity, character, and duration of the noxious stimuli.

15. Relationship to Other Projects:

The two BNL strains of rats are unique, and not generally available. Therefore duplication of this research is unlikely. However, two other strains of rats that develop "spontaneous" hypertension are now available. The first of these was reported by Smirk in 1958 and the second by Okamoto and Aoki in 1963. Smirk's rats have not been generally available whereas those of Okamoto and Aoki are now in use throughout the world. In both of these strains, environmental influences appear to be much less important than in the BNL strains. It is impractical to summarize the extensive work on the Japanese rats (called SHR for "spontaneously hypertensive rat"), in order to show there is no substantive overlap of the work at BNL and at other labs. A monograph on the SHR that was published in 1972 based on the proceedings of a conference in Kyoto, 18-22 October 1971, provides the most complete summary extant of work based on the SHR model and it is clear that there is no significant duplication of the efforts at BNL.

(See Continuation Sheet)

15. Relationship to Other Projects: (Cont'd.)

Freis at the VA Hospital in Washington, D. C. is studying the influence of dietary NaCl on the blood pressure of the SHR from the standpoint of modifying BP by anti-hypertensive drugs.

16. Technical Progress in FY 1973:

The effect of renal transplants on the chronic blood pressure response was studied in the two strains of rats with opposite genetic propensities to hypertension. 113 rats survived an average of 4 months (range 1-14) after this procedure. Among animals maintained on a low NaCl diet, blood pressure was not significantly affected when the recipient animal and its renal homograft came from the same strain; however, animals from the hypertension-resistant strain with a renal homograft from the hypertension-prone strain had higher pressures whereas hypertension-prone rats with a homograft from the hypertension-resistant strain had lower pressures than their respective controls. Thus, the phenotypic response--blood pressure--was more influenced by the genotype of the renal homograft than by the genotype of the recipient. The continuing work with renal homografts has demonstrated, for the first time, that at least one of the genetically determined influences on blood pressure resides in the kidney. This technically difficult study is being actively pursued.

The influence of renal autografts on BP is being studied to determine whether subtle sub-clinical rejection may be occurring. Among animals from the S strain with salt-induced hypertension, it appears that kidneys from the hypertension-resistant rats lower the BP of such hypertensive recipients whereas kidneys from the hypertension-prone strain either result in no change or a further elevation in hypertensive recipients. These data, then, are compatible with the initial experiment obtained in non-hypertensive recipients, i.e., the kidneys from the R strain tend to lower BP to normal levels whereas kidneys from the S strain tend to raise BP above normal.

In continuation of the study on influence of renal parenchymal injury on BP as modified by genetic background, the influence of prolonged anesthesia (animals were anesthetized for approximately 3 hours) and, in right uninephrectomized rats, of anoxic injury produced by clamping the left renal artery for 1 hour, were explored in animals not subjected to renal transplant operations. These data are being analyzed statistically but the preliminary impression is that: a) anesthesia is without effect but, b) anoxia for one hour combined with added dietary NaCl results in some hypertension even among rats ordinarily resistant to it. This could have significant clinical implications among individuals with various degrees of impaired renal blood flow or among the increasing number of people who receive renal homografts which will have necessarily been anoxic for a variable period prior to and during surgery. It would also aid in explaining why salt-fed R rats with R renal homografts or autografts sometimes develop hypertension.

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

Rabbit antikidney serum (AKS) induced hypertension in S rats but not in R, in apparent confirmation of the hypothesis suggested in Section 14B. Histological examination, however, showed lesions compatible with AKS nephritis in S rats but not in R. In the absence of evidence of nephritis in R rats, therefore, it cannot safely be concluded that the hypothesis has been confirmed. The failure of R rats to show lesions may be only accidental although it stretches probability to so explain it. A more exciting possibility is that the AKS is not allergenic for R rats. Studies are being repeated.

Efforts to induce experimental pyelonephritis so far have failed. Further attempts will be made in collaboration with the new staff Bacteriologist, Dr. Pavlova.

Studies on the renin-angiotensin system in the two strains of rats continued. S rats had significantly lower activities of plasma and renal renin than R rats on four experimental regimens: low NaCl diet, high NaCl diet, unilateral renal artery constriction, and unilateral renal artery constriction plus contralateral nephrectomy. It was concluded that renin activities, like blood pressure, were modified by genetic influences. Previous observations suggested that the plasma of S rats contains a factor which inhibits the kidney enzyme (renin) responsible for the release in plasma of a hypertensive substance (angiotensin). Since this inhibitor was not observed in plasma from the very closely related, but hypertensive-resistant R strain, it seemed likely that this factor might be involved in the development of hypertension. Further investigation using radio-immunoassay of angiotensin formed by renin in plasma has indicated that renin is not inhibited by plasma from hypertension-prone rats. The apparent conflict with the earlier observations is attributed to the different methods used for assay--the radioimmunoassay measures angiotensin I directly whereas the bioassay measures angiotensin I indirectly through the pressor response to angiotensin II produced from angiotensin I enzymatically in the test animal. Current studies are directed toward establishing whether the factor present in hypertension-prone rats is itself hypotensive or reduces the response to angiotensin.

In the collaborative studies of renal function in the R and S rats with Dr. Ben-Ishay the diuretic and natriuretic responses to acute oral salt loads were studied. Hypertonic saline loads elicited similar responses in the two strains, but isotonic loads produced significantly higher salt and water excretion in S rats. These data suggest an abnormal renal concentrating mechanism and were interpreted as being compatible with a defect in Henle's loop in S rats. This study indicates for the first time that sodium is handled differently by the kidneys of the two strains. Since there is increasing evidence that sodium is involved in all forms of hypertension this may be a crucial observation.

(See Continuation Sheet)

Nuclear Medicine Technology and Other Health Applications
Interrelationsip between Genetic and Environmental Factors
Project Title: in Clinical and Experimental Hypertension RX-01-03-(b)

16. Technical Progress in FY 1973: (Cont'd.)

Studies on glomerular filtration rate with C-14 inulin and on renal blood flow with para-amino-hippurate were performed on R and S rats with renal homografts from either strain; as compared with similar studies on appropriate controls, normal renal function was observed in the animals with homografts for up to ten months--the longest period studied.

Studies on corticosteroid production and genetic effects on blood pressure in the salt susceptible and salt resistant strains of rats were completed in collaboration with Dr. John Rapp. Classical quantitative genetic techniques suggest that the genetic component of blood pressure in these strains is controlled by relatively few genetic loci, i.e., on the order of 2-4. One such locus with 2 alleles inherited by co-dominance was identified which controls adrenal output of 18-hydroxy-deoxycorticosterone (18OH-DOC), a hypertensinogenic mineralocorticoid. This locus accounts for approximately 16% of the blood pressure difference between S and R strains with the remaining 84% due to other, unidentified genes.

Studies on the influence of dietary potassium and Na/K molar ratios on the development of salt hypertension were completed. Among genetically hypertension-prone rats, dietary sodium (chloride) was demonstrably hypertensinogenic and potassium (chloride) antihypertensinogenic. On diets containing the same NaCl but different KCl concentrations, mean blood pressure was greater in rats receiving less dietary potassium, i.e., diets with a higher Na/K molar ratio. On diets with different absolute concentrations of NaCl and KCl, but the same Na/K molar ratios, rats on the higher absolute NaCl intakes had the higher blood pressures. On diets with different absolute concentrations of NaCl and KCl, and different Na/K molar ratios, a group on a lower absolute NaCl intake but with a higher Na/K ratio could have more hypertension than a group on a higher absolute NaCl intake but with a lower Na/K ratio. At equivalent molar ratios, the respective effects of these two ions on blood pressure were dominated by that of sodium. It was concluded that the dietary Na/K molar ratio can be an important determinant for the severity, or even development, of salt-induced hypertension. The mechanism of the moderating effect of potassium on sodium-induced hypertension is unclear.

A genetic study involving a cross between the BNL S rats and the Japanese spontaneously hypertensive rats (SHR) was initiated in an effort: a) to determine what genes are shared in common, and b) to improve the estimate of the number of genes involved in the R and S strains. F_1 rats off and on NaCl are being bred for backcross and F_2 generations and the BP response observed. Early study of SHR on high and low NaCl diets shows that SHR and F_1 rats on NaCl develop hypertension more rapidly and die earlier than comparable animals on low NaCl.

Adrenal transplant studies were reactivated. About 50 successful transplants were performed (confirmed at autopsy after death). Inspection of the data suggests that no major change in the usual evolution of blood

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

pressure occurred. This might be predicted from calculations published earlier showing that the difference in 18OH-DOC production accounts for only about 15% of the difference in blood pressure between the two strains. Further studies depend on the availability of Dr. Rapp (University of Pennsylvania) to undertake a study of steroidgenesis in the transplanted adrenals.

Clinical observations suggest a possible cause-effect relationship between the use of contraceptive steroids (CS) and the development of enhancement of high BP in some women. In conjunction with Dr. Woods (University of North Carolina) this was studied in S rats. CS enhanced the hypertension of rats receiving a high salt diet but not those on low NaCl. This is the first successful animal model for study of this problem.

Most female S rats develop hypertension more slowly than males although ultimately attaining the same levels. A long-standing clinical debate concerns the influence of the menopause on BP. In studying the influence of gonadectomy and replacement therapy on the development of hypertension, gonadectomy was found to be without influence on the BP of the male whereas in the female it led to a BP pattern indistinguishable from the male. As this suggests the possibility that pre-hypertensive females may develop hypertension more rapidly after the menopause further studies are in progress.

With the collaboration of Friedman (State University of New York at Stony Brook) the study of "stress" and hypertension was reactivated. The method of "operant conditioning" is used which requires the animal to learn to press a bar in order to obtain food but during the course of which the animal may also receive punishment (electroshock). The number of bar pressings required to receive both a unit of food and electroshock is randomly varied (within limits set by the operator) and the two are not correlated so that neither can be predicted by the test animal. This is currently considered to be the most stress-provoking experimental technique available. Although previous attempts here were unsuccessful in producing hypertension in rats using other stress-provoking techniques, there is so much clinical evidence suggesting that "stress" can elevate BP in man, that this additional attempt to induce hypertension experimentally with "stress" was initiated.

Hypertension accelerates the development of atherosclerosis and atherosclerosis causes most of the complications from which hypertensives die. About half of the patients with myocardial infarcts have pre-existing hypertension. For these reasons efforts are being made to develop animals genetically predisposed and resistant to both hypertension and hypercholesterolemia. The R and S strains are being used to develop two substrains from each of which one substrain will be genetically predisposed to develop a low, the other a high, serum cholesterol while on the same high fat-cholesterol

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

diet. If successful one could study the interactions of hypertensino-and-atherogenic influences experimentally in a fashion never before possible. After 5-6 generations of inbreeding, 2 substrains from R and S strains are emerging with trends towards low and high plasma cholesterol. Exceptions appear, however, indicating that multiple genes are involved and that it may take longer to separate the substrains than anticipated.

There is evidence that some human hypertensives fail to suppress aldosterone adequately in response to increased salt intake resulting in inappropriately high aldosterone levels for such salt intakes. Aldosterone production and zona glomerulosa size were studied in the S and R rats in relation to salt intake in collaboration with Dr. John Rapp. With either *in vitro* or *in vivo* systems S rats suppressed aldosterone production in response to salt as much or more than R animals. Zona glomerulosa mass was not different between S and R rats on various salt intakes. It was concluded that S rats are able to normally suppress aldosterone in response to salt in spite of their genetically controlled increment in adrenal 18-hydroxylase activity reported previously.

The study on radiation-genetic interaction in experimental hypertension is dormant. The initial study was completed but incompletely analyzed: the absence of Dr. Knudsen, effectively since early Spring 1972, has slowed this work.

Circumstantial evidence suggests that "overeating" promotes and "undereating" retards manifestations of hypertension, atherosclerosis, diabetes, gout, and obesity. Each may present varying degrees of hypertension, hypertriglyceridemia, hypercholesterolemia, hyperuricemia, hyperlacticacidemia, glucose intolerance, and insulin insensitivity. Patients with one of the diseases frequently develop one or more of the others. Although the mode of inheritance is not known the genetic factors are probably multi-factorial. In benign forms the diseases are usually found in middle or old age whereas malignant forms may occur in youth. It is proposed that the association among the five diseases above indicates that they share some genetically determined biochemical defects in which the character of the clinical manifestations will be influenced by the nature of the input from the environment, i.e., in a sense, the kind of "overeating".

In 68 hypertensive patients 25% had a strongly abnormal glucose tolerance curve, 25% had a borderline abnormal response, and 50% were normal, whereas non-hypertensive controls were 0, 25, and 75% respectively. These differences were significant after correction for age, weight, and sex. Blood insulin levels after a glucose load determined by immunoassay have been reported by another group to be abnormally high in hypertensives. In the 68 BNL hypertensives 35, 43, and 22% had "high", "borderline", and

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

"normal" insulin levels contrasted with 26, 18, and 56% in the controls. After correction for age, weight, and sex, the differences were not significant. It is concluded that there is a signficant association between hypertension and reduced glucose tolerance but probably not an increased insulin response to a glucose challenge in these same patients.

A recent study of the 24-hour excretion of uric acid in patients on a constant low-purine diet indicates that hypertensives have an increased excretion of uric acid not explicable on changes in renal uric acid clearance but probably reflecting a larger-than-normal "pool" of uric acid.

17. Expected Results in FY 1974:

Kidney transplant studies will continue. Injection of antikidney serum (AKS) will be repeated to see if AKS nephritis can be provoked in R rats. Also the experimental pyelonephritis study will be repeated as soon as Dr. Pavlova can help with the bacteriology. The initial adrenal transplant study will be completed.

Study of renin activities in plasma of rats with renal homo- and autografts will be expanded. The preliminary exploration of the usefulness of operant-conditioning as a technique for inducing hypertension should be completed allowing decision as to feasibility of expanding or discontinuing the study. A new study of uric acid pools in human hypertension will be initiated.

Exploration of the renin-angiotensin system will continue, in particular with reference to the factor that modified the in vivo response to angiotensin. The study of effect of NaCl on Japanese "spontaneously hypertensive rats" and the study of influence of gonadectomy on BP should be completed.

Arrangements have been completed to begin kidney micropuncture studies some time during the next year in the laboratory of Dr. Alain Grandchamp of the Department of Medicine at the University of Geneva Medical School. Such studies may be critical in understanding the recent finding that sodium is handled differently by the kidneys of the two strains and may provide fundamental insight into the pathogenesis of hypertension.

18. Expected Results in FY 1975:

Studies will continue as dictated by findings in the previous fiscal year.

19. Description and Explanation of Major Materials, Equipment, and Subcontract Items:

None

(See Continuation Sheet)

Project Title: Nuclear Medicine Technology and Other Health Applications
Interrelationship between Genetic and Environmental Factors
in Clinical and Experimental Hypertension RX-01-03-(b)

20. Proposed Obligations for Related Construction Projects:

None

(See Continuation Sheet)

Nuclear Medicine Technology and Other Health Applications
Interrelationship between Genetic and Environmental Factors
Project Title: in Clinical and Experimental Hypertension RX-01-03-(b)

Reiteration of Assurance Statement
on
Investigation Involving Human Subjects
Including Clinical Research

The Hospital of the Medical Research Center, Brookhaven National Laboratory, complies with the policy for the protection of human subjects participating in activities supported directly or indirectly by grants or contracts from the Department of Health, Education, and Welfare and from the Atomic Energy Commission. In fulfillment of its assurance, this institution has established a standing committee to review investigations involving human subjects, including clinical research, and said committee is charged with responsibilities to determine for each proposed study that:

the rights and welfare of each patient will be adequately protected,
that the potential benefits outweigh the risks, and
that an informed consent will be obtained from each patient.

The committee, appointed by the Director, Brookhaven National Laboratory, provides for independent judgment of its members, and alternates are provided to serve when there may be conflict of interest. This institution encourages constructive communication between the committee and principal investigators on the safeguarding of the rights and welfare of subjects; will provide the required care for persons injured as a result of participation; and maintains complete documentation of proposals and reviews. Approved investigations are reviewed by the committee at least annually and the institution periodically reviews its practices and procedures to assure they are consistent with the policies published by the Department of Health, Education, and Welfare.

As of March 7, 1973, the Institutional Review Committee for review of Medical Department research activities involving human subjects is composed of:

J. S. Robertson, M.D., Chairman
G. C. Cotzias, M.D., Alt. Chairman
S. H. Cohn
E. A. Popenoe, Alt. for Dr. Cohn
G. Chikkappa, M.D.
H. Connell
R. A. Love, M.D.
G. Price
N. P. Rathvon, Jr.
D. N. Slatkin, M.D., Alternate
A. P. Wolf, Alternate
L. K. Dahl, M.D. - Clinical Consultant
W. A. Finn - Administrative Consultant
C. W. Flood - Health Physics Consultant

Signature: Eugene P Cronkite

Title: Chairman, Medical Department

Date: March 7, 1973

RX-55

1179213

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory — RX-Biomedical & Environmental Research
Laboratory — Program

1. Contractor: Associated Universities, Inc. — Contract No.: AT(30-1)-16 — Task No.:

2. Project Title: Nuclear Medicine Technology and Other Health Applications Extension and Improvement of Radiographic and Isotopic Diagnostic Techniques — 189 No.: RX-56

3. Budget Activity No.: RX-01-03-(c)

4. Date Prepared: May 1973

5. Method of Reporting:
Scientific Meetings
BNL Monthly Letter to AEC
Scientific Journals

6. Working Location: Brookhaven National Laboratory

7. Person in Charge:
H. L. Atkins
L. V. Hankes (FY 1973 only)

Principal Investigator:
H. L. Atkins
P. Richards (Dept. of Applied Science)
A. P. Wolf (Chemistry Department)
H. Kraner (Instrumentation Division) L.V. Hankes (FY 1973 only)

8. Project Term: Continuing — From: — To:

9. Man-Years:

Direct Man-Years	FY 1973	FY 1974	FY 1975
Scientific & Professional	10.0	7.5	6.5
Other	14.5	14.5	15.5
Guests & Res. Collaborators	2.0	3.0	3.0
Total	26.5	25.0	25.0

10. Costs (In Thousands of Dollars):

	FY 1973	FY 1974	FY 1975
Research Division	575	497	570
Hospital Division	138	213	225
Research Costs	713	710	795
Equipment Obligations	37	83	110

11. Reactor Concept:

12. Materials:

Project Title: Nuclear Medicine Technology and Other Health Applications
Extension and Improvement of Radiographic and Isotopic Diagnostic Techniques RX-01-03-(c)

13. Publications:

Robertson, J. S., Fairchild, R. G. and Atkins, H. L. Dosimetry of californium 252. Radiology 104, No. 2, 393-8 (1972). 16332

Fowler, J. S. A new synthesis of unsymmetrical azo compounds. J. Org. Chem. 37, 510 (1972). 16021

Atkins, H. L., Christman, D. R., Fowler, J. S., Hauser, W., Hoyte, R. M., Klopper, J. F., Lin, S. S. and Wolf, A. P. Organic radiopharmaceuticals labeled with isotopes of short half life. V. ^{18}F-labeled 5- and 6- fluoro-tryptophan. J. Nucl. Med. 13, 713-9 (1972). 16382

Atkins, H. L., Eckelman, W. C., Hauser, W., Klopper, J. F. and Richards, P. Splenic sequestration of ^{99m}Tc-labeled red blood cells. J. Nucl. Med. 13, No. 11, 811-4 (1972). 16623

Hankes, L. V., Leklem, J., Brown, R. R., Mekel, R. C. and Jansen, C. R. Abnormal tryptophan metabolism in bantu with scurvy-type skin. Biochem. Med. 7, 184-9 (1973). 16685

Atkins, H. L. and Klopper, J. F. Measurement of thyroidal technetium uptake with the gamma camera and computer system. J. Nucl. Med. (in press). 16745

Atkins, H. L., Eckelman, W. C., Klopper, J. F. and Richards, P. Vascular imaging with ^{99m}Tc-red blood cells. Radiology (in press). 16733

Atkins, H. L., Fairchild, R. G. and Drew, R. M. Biological properties of ^{252}Cf. Am. J. Roentgenol. Radium Therapy Nucl. Med. (in press). 16873

Atkins, H. L., Klopper, J. F., Lambrecht, R. M. and Wolf, A. P. A comparison of technetium-99m and iodine-123 for thyroid imaging. Am. J. Roentgenol. Radium Therapy Nucl. Med. (in press) 16932

Atkins, H. L., Klopper, J. F., Eckelman, W. C. and Richards, P. The technetium-99m-DTPA renal study. Presented at the IAEA Symposium on Radioisotope Scintigraphy, Monte Carlo, October 1972. 17032

14. Scope:

A) 200 Word Summary:

There are two main objectives in the various studies carried on in this budget activity: development of diagnostic agents for clinical application to human diseases; and reduction of radiation dosage in nuclear medicine examinations. The major health problems in which nuclear medicine techniques can improve diagnosis and reduce radiation exposure are heart, pulmonary and neoplastic diseases. The studies, centered on radiopharmaceutical development, necessitate extensive interdisciplinary collaboration.

(See Continuation Sheet)

Project Title: Nuclear Medicine Technology and Other Health Applications
Extension and Improvement of Radiographic and Isotopic Diagnostic Techniques RX-01-03-(c)

14. Scope:

B) Supplement to 200 Word Summary:

The leading cause of death in the United States is cardiovascular disease--death rate, 350-400 per 100,000 population--and thus major efforts are directed at its prevention and treatment. The development of operative procedures to improve coronary blood flow requires assessment of myocardial blood flow in order to select patients for surgery. Since the patients are critically ill, the procedure must be non-traumatic. Nuclear medicine techniques are non-invasive and gentle and particularly advantageous when they can be made universally available. One proposal in these studies is development of methods for myocardial scanning using thallium 201, a potassium analogue with a low energy gamma emission, readily detected and collimated and with a half-life of 3.08 days. A promising radionuclide for frequent sequential scanning is rubidium 82, a positron emitting analogue of potassium with a half-life of only 75 seconds. Also, a high early concentration of carbon-11 norepinephrine has been observed in the mouse heart, suggesting that it could be developed for myocardial imaging in some circumstances.

One of the causes of heart disease is hypertension and among the causes of hypertension are diseases of the adrenals. Although methods for localizing adrenal cortical lesions exist, a research effort here is to develop a technique using carbon-11 dopamine for adrenal medullary imaging to diagnose pheochromocytoma.

Chronic, obstructive pulmonary disease (COPD), a major cause of death, is reversible if diagnosed early. To date, no method has been developed for its detection in asymptomatic individuals. In collaboration with members of the BNL Department of Applied Science, a simple test, adaptable to mass screening of populations is being developed. After detection of early disease by screening, assessment of regional ventilation and perfusion for localization of the disease process is important. Thus, methods are studied using BLIP (Brookhaven Linac Isotope Producer) produced Xe-127 to replace the rather ineffective Xe-133 presently in use.

The second leading cause of death in this country is cancer, and early diagnosis is essential to effective treatment. Mammography aids early diagnosis of mammary cancer. X-ray mammography is the present technique used, yet it is unsuitable for mass screening of the female population because it gives a high radiation dose to the skin and poor contrast between tumor and normal tissue. Tc-97m has the appropriate gamma energy for maximum contrast and would reduce the skin radiation dose by a factor of 20. Tc-97m, produced in BLIP and fabricated into appropriate sources will be applied in mammography.

Another cancer problem is the diagnosis of intra-ocular melanomas. In collaboration with an opthalmologist, and using specially-designed detectors and collimators made by the members of the BNL Instrumentation Division and Department of Applied Science, I-123 labeled quinoline

(See Continuation Sheet)

1179216

14. Scope: (Cont'd.)

derivatives will be studied for localization of ocular and, probably, other melanomas.

The gonadal radiation dose to the population at large from radioisotopic procedures used in Nuclear Medicine today, though still acceptably small, has increased greatly over the past ten years. If the trend continues, the dose incurred by the population at large could become significant. Therefore, development and use of short-lived radionuclides which can provide the same or better information at a lower radiation dose is emphasized. I-123 is an example. When used for thyroid diagnosis, the gonadal dose is 1/100 of that from I-131. Thyroid disease is common in women of child-bearing age. Studies on application of I-123 will be extended with its replacement of I-131 and I-125 as labels for other radiopharmaceuticals. (Atkins)

The amino acid, tryptophan, produces ortho-amino-phenol type compounds believed to induce cancer in animal urinary bladders. In several types of cancer and in other diseases of man, the literature reports significant increases in urinary levels of some tryptophan metabolites. Carbon-14 labeled metabolites are used in animals and man to determine the normal tryptophan metabolic pathway. The labeled metabolites include anthranilic acid, kynurenine, hydroxykynurenine, and hydroxyanthranilic acid. Diseases studied include anemias, scleroderma, siderosis, malaria, pellagra, and various cancers. These studies will be reported for FY 1974 and 1975 in RX-01-03-(e). (Hankes)

15. Relationship to Other Projects:

Similar investigations involving radiopharmaceuticals and cyclotron-produced nuclides are carried out at many institutions. However, BNL is in an exceptional position due to the presence of several high energy accelerators of unusual capabilities as well as an interdisciplinary cooperative program including the Medical Department, Chemistry Department, Department of Applied Science, and the Instrumentation Division. Particular expertise exists here which would be difficult to find in all these fields in any one other institution. (Atkins)

Research projects related to the tryptophan studies are detailed in Section 15 of RX-01-03-(e).

16. Technical Progress in FY 1973:

Technetium-labeled red blood cells were evaluated for splenic imaging and showed promise for blood pool and vascular imaging. Tc-99m-DTPA is now in routine use for renal studies and is being compared to I-131 hippuran for evaluation in hypertension.

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

I-123 was compared with Tc-99m for thyroid imaging; the definite superiority of I-123 was apparent. Labeled hippuran was also used but sufficient quantities are not now available for clinical use. A study of I-123 iodoquin for eye melanomas is in progress in collaboration with Dr. Samuel Packer of St. Albans Naval Hospital, the BNL Chemistry Department and Instrumentation Division.

Evaluation of carbon-11 labeled dopamine for adrenal scanning has shown excellent concentration of the compound in the adrenal medulla as compared to other tissues at two hours. However, this is impractical because of the short half-life of C-11. Relative concentrations at earlier times are being evaluated.

Thallium-201 production methods are being worked out on the cyclotron for use prior to BLIP production of the isotope. Myocardial localization has been determined in mice and rats. The ability to visualize infarcted areas in the myocardium is being evaluated in goats following surgical induction of infarcts by ligating coronary arteries.

In the studies on transmission scanning, the Instrumentation Division has devised a useful clinical system for imaging iodine distribution in vivo. With a radiation dose of only a few mrad, it should be possible to image the iodine naturally found in the thyroid. Animal studies showed the distribution of Cholografin and Hypaque, iodinated contrast agents in the biliary system and urinary tract. Xenon also was used successfully as a positive pulmonary contrast agent in animal studies. Use in humans awaits approval of the Institutional Review Committee of the Hospital of the Medical Research Center.

A contract with NIH for production of Tc-97m as a radiographic source for mammography was approved and funded. The use of critical absorption edge filters to improve radiographic contrast, possibly resulting in reduced radiation exposure, is being evaluated.

The simplified helium early closure test of pulmonary function using helium and a leak detector mass spectrograph was applied to a large number of normal individuals at all age levels and with varying smoking habits. The apparatus developed in collaboration with members of the BNL Department of Appli[redacted] Science determines the time of small airway closure, the earliest indi[redacted]on of chronic obstructive pulmonary disease. The results are being analy[redacted]. (Atkins)

The following report of progress during the past year is repeated in RX-01-03-(e).

(See Continuation Sheet)

Project Title: Nuclear Medicine Technology and Other Health Applications
Extension and Improvement of Radiographic and Isotopic Diagnostic Techniques RX-01-03-(c)

16. Technical Progress in FY 1973: (Cont'd.)

A metabolic study of the L-isomers of carbon-14 labeled tryptophan, kynurenine, and hydroxykynurenine in patients with scleroderma was conducted at the F. W. Verwoord Hospital in Pretoria, South Africa. The purpose of the study was to determine whether the South African type of scleroderma had metabolic abnormalities similar to those of the American type. The South African scleroderma patients were male caucasian miners whose illness began with silicosis followed by later development of scleroderma. Breath carbon dioxide samples were collected in sodium hydroxide over a 24-hour period after the C-14 labeled doses were given and 24-hour urine samples were collected for a three day period. The breath $^{14}CO_2$ level for those given tryptophan-7a-C-14 was 6.3 - 8.9 percent; L-hydroxykynurenine-keto-C-14 was 30.6 - 37 percent and L-kynurenine-keto-C-14 was 48.6 - 63.3 percent. The values are similar to those found in the American scleroderma patients given the same compounds. Kynurenine, hydroxykynurenine, xanthurenic acid, kynurenic acid, picolinic acid, quniolinic acid, N-methylnicotinamide, N-methyl-2-pyridone-5-carboxamide and nicotinic acid will be isolated from the urine by carrier techniques and the carbon-14 content of these urinary components determined. The results from these analyses should help determine the particular metabolic abnormalities in males with this disease. This long range project has made little progress due to insufficient technical assistance. The South African Atomic Energy Board (SAAEB) is sending Dr. E.J.P. de Bruin to Brookhaven to spend a year of post-doctorate study at their expense and to help with the collaborative projects. Dr. de Bruin will work on the scleroderma, pellagra, and hepatic tumor problems.

The tryptophan metabolic studies in prisoners with a sensitivity to primaquin drugs in the treatment of malaria, were expanded to include a study of kynurenine metabolism. In collaboration with Dr. Carson at Stateville Prison, Illinois, urine samples were collected from five prisoners before and after loading with tryptophan. The urines were analyzed for 16 tryptophan metabolites to provide control values for other studies of the American Negro and African Bantu. Some subjects were given 400 mg doses of carbon-14 labeled L-kynurenine and the study requires several more subjects receiving 200 mg doses.

Observations reported last year suggest that South African pellagrins have subnormal vitamin B_6 coenzyme levels in addition to the previously proposed stress-induced increase in the activity of tryptophan pyrrolase or a lack of feedback control of this enzyme by low pyridine nucleotides. A gra[illegible] (strongly recommended by the Japanese-American Study Commission) has b[illegible] obtained from the DHEW to provide two technicians to continue this internationally important project.

In 1972 a limited number of pellagra patients were studied during the pellagra season in South Africa. At the request of Dr. Roux, President, and Dr. Jansen, Head of the Life Sciences Division, SAAEB, the study will

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

be completed in 1973 with travel and laboratory expenses paid by SAAEB. In addition, patients with primary hepatoma will also be studied. This hepatoma is nutritionally based, causes death within three months, and has a mortality rate of more than 150 per hundred thousand per year among the Bantu.

A BNL research collaborator, Dr. Ludwig Feinendegen, Director of The Institute for Medicine, KFA, Julich, Germany, has requested a week's visit by the Principal Investigator to set up a collaborative study at Julich utilizing double labeled tryptophan. (Hankes)

17. Expected Results in FY 1974:

Collaborative studies with the Department of Applied Science will continue on technetium labeled compounds, pulmonary function, indium 111, myocardial agents and radiography.

Erythrocytes labeled with Tc-99m have been used for vascular and splenic imaging. One detraction from widespread clinical use is the 60-70% efficiency of labeling which requires two cell washes. However, there are indications that labeling efficiency can be increased to over 90%. This will greatly simplify the procedure and make it more practical.

The helium leak detector test determining time of airway closure in health and disease will be extended to a larger population group to determine normal parameters. It is planned to follow up employees at yearly intervals as a longitudinal study and to compare smokers, non-smokers, and former smokers. When abnormalities are detected, regional ventilation will be studied with radioactive gases. Xenon 133 can be used, but when Xenon 127 is available, this will be evaluated.

The parameters of *in vitro* labeling of transferrin with indium 111 will be studied and distribution quantified in animals. Plasma clearance, excretion, and distribution in human beings will be studied and compared to technetium-sulfur colloid. When iron 52 is available, similar comparative studies will be carried out. Thallium 201 will be evaluated further as a myocardial scanning agent. Studies will be continued in goats to determine degree of myocardial localization as well as patterns of distribution in other tissues. Data will be used for dosimetry purposes. Thallium 201 also localizes in kidneys and the concentration, time of peak localization, and rate of disappearance will be determined in animals and patients. There is evidence to suggest that thallium may be useful as a tumor localizing agent, particularly in melanomas. This will be tested in a hamster melanoma system.

(See Continuation Sheet)

17. Expected Results in FY 1974: (Cont'd.)

In addition to work under the NIH contract investigating techniques for mammography, spectral measurements of radiographic tubes will be performed and the use of critical absorption filters for mammography and iodinated contrast agents will be studied in order to evaluate possible reduction in dose and increase in contrast.

Studies in collaboration with the Chemistry Department will include iodine 123, carbon 11 and F-18 fluorinated amino acids. Thyroid imaging with orally administer I-123 will be evaluated using a smaller intravenously administered dose with dynamic studies of rate of immediate uptake of iodine utilizing a computer system. The ocular study with labeled 4-[3-dimethyl-aminopropylamino]-7-iodoquinoline(DMQ) will be continued in collaboration with Dr. Samuel Packer and extended to human patients. Comparative studies using ultrasound and P-32 should be carried out. Other iodinated agents should be evaluated, chiefly I-123 hippuran for renography, utilizing the MED II computer for analysis. Rose Bengal should also be useful for biliary problems.

Dopamine, labeled with C-11, has been produced in carrier free form. The degree to which it localizes in the adrenals is highly dependent on the specific activity and that of the carrier free material is from five to seven times better than our previous best material (40-50 mCi/mg). Following further quantitation in dogs, attempts will be made to visualize the human adrenals. If successful, an evaluation of hypertensive patients would be indicated. There are also possibilities of C-11 dopamine localizing in specific areas of the brain, particularly in combination with the administration of monoamine oxidase inhibitors. Studies are to be performed first on mice, then on dogs, with the aim of eventual studies of patients with parkinsonism. Other biogenic amines including norepinephrine and epinephrine will be studied. An eventual goal is the labeling of L-dopa with C-11 and obtaining information concerning its disposition and metabolism in Parkinson's disease patients.

F-18 labeled fluorotryptophan has been evaluated in animals and appears to hold promise for imaging of the pancreas. As soon as methods for assuring sterile and pyrogen free materials have been developed, application to human disease is envisaged.

In collaboration with the Instrumentation Division work will continue on transmission scanning apparatus, the positron camera, and semiconductors. An absorption edge transmission scanning apparatus, developed and tested using phantoms and animals, appears to be useful for plotting and quantifying distribution of iodine in vivo in the thyroid or in other areas such as the biliary or urinary tracts after injection of iodinated contrast agents. Application to patients will be started. All patients referred for thyroid work-up will have transmission scans. Problems of the biliary tract will be studied with injection of iodipamide. Further instrumentation development

(See Continuation Sheet)

17. Expected Results in FY 1974: (Cont'd.)

is envisioned, particularly interfacing with the MED II computer for easier quantification. Interfacing of the positron camera for use with C-11 and F-18 compounds is planned. This will also require a major effort in computer programming.

A high purity germanium detector is in use for bone mineral analysis and for transmission scanning, both of which were developed by our Instrumentation Division. A semiconductor camera device would be extremely useful in conjunction with transmission scanning and for use with Tc-99m and I-123 compounds. (Atkins)

18. Expected Results in FY 1975:

Studies described above will continue, with specific emphasis determined by instrumentation and radiopharmaceuticals developed during the preceding year.

19. Description and Explanation of Major Materials, Equipment, and Subcontract Items:

FY 1975 Capital Equipment:

The major capital requirement is an image intensifier and video disc recorder in order to do correlative animal studies for investigations of radiopharmaceuticals in coronary heart disease. This would permit coronary angiography to determine patency of arteries prior to and after inducing infarctions in large animals as a control for radionuclide imaging procedures. The apparatus would be useful also for routine veterinary x ray, and for angiography of renal transplants ($90,000).

20. Proposed Obligations for Related Construction Projects:

None

(See Continuation Sheet)

Project Title: Nuclear Medicine Technology and Other Health Applications
Extension and Improvement of Radiographic and Isotopic
Diagnostic Techniques RX-01-03-(c)

Reiteration of Assurance Statement
on
Investigation Involving Human Subjects
Including Clinical Research

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D. N. Slatkin, M.D., Alternate
A. P. Wolf, Alternate
L. K. Dahl, M.D. - Clinical Consultant
W. A. Finn - Administrative Consultant
C. W. Flood - Health Physics Consultant

Signature: Eugene P Cronkite

Title: Chairman, Medical Department

Date: March 7, 1973

RX-65

1179223

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory — Laboratory

RX-Biomedical & Environmental Research — Program

1. Contractor: Associated Universities, Inc.
Contract No.: AT(30-1)-16
Task No.:

2. Project Title:
Nuclear Medicine Technology and Other Health Applications
Lymphocytopoiesis and Transplantation Immunology
189 No.: RX-66

3. Budget Activity No.: RX-01-03-(d)

4. Date Prepared: May 1973

5. Method of Reporting:
Scientific Meetings
BNL Monthly Letter to AEC
Scientific Journals

6. Working Location:
Brookhaven National Laboratory

7. Person in Charge:
E.P. Cronkite

Principal Investigator:
E.P. Cronkite D.D. Joel
A.D. Chanana G. Chikkappa
I. Laissue

8. Project Term:
Continuing
From: To:

9. Man-Years:

Direct Man-Years	FY 1973	FY 1974	FY 1975
Scientific & Professional	5.5	6.5	6.0
Other	12.5	12.5	16.0
Guests & Res. Collaborators	2.5	4.0	4.0
Total	20.5	23.0	26.0

10. Costs (In Thousands of Dollars):

	FY 1973	FY 1974	FY 1975
Research Division	374	407	446
Hospital Division	104	152	181
Research Costs	478	559	627
Equipment Obligations	50	16	55

11. Reactor Concept:

12. Materials:

Nuclear Medicine Technology and Other
Health Applications

Project Title: Lymphocytopoiesis and Transplantation Immunology RX-01-03-(d)

13. Publications:

Joel, D. D., Hess, M. W., and Cottier, H. Massive migration of thymic lymphocytes to Peyer's patches in neonatal mice. Morphological and Functional Aspects of Immunity. Lindahl-Kiessling, G. Alm and M. G. Hanna, Editors, pp. 141-7, Plenum Press, New York, 1971. 16576

Van Bekkum, D. W., and Cronkite, E. P. Radiation effects on the lymphoid systems and its functions. Progress in Immunology, pp. 1205-9, Academic Press, Inc., New York and London, 1971. 16583

Joel, D. D., and Cronkite, E. P. Extracorporeal irradiation of blood and lymph. Presented at National Conference on Research Animals in Medicine, Washington, D. C., January, 1972. 16579

Burlington, H., and Cronkite, E. P. Characteristics of renal glomeruli in culture. Exp. Cell Res. (in press). 16635

Chanana, A. D., Joel, D. D., Schaedeli, J., Hess, M. W., and Cottier, H. Thymus cell migration: ^{3}HTdR-labeled and THETA-positive cells in peripheral lymphoid tissues of newborn mice. Presented at the 4th International Conference on Lymph, Tissue and Germinal Centers in Immune Reactions, Dubrovnik, Yugoslavia, June, 1972. 16952

Cronkite, E. P. Radiosensitivity of lymphocytes. Presented at the Conference on Radiosensitivity, Freiburg, Germany, September, 1972. 17253

Chikkappa, G., Boecker, W. R., Borner, G., Carsten, A. L., Conkling, K., and Cook, L. Bone marrow (BM) cells of chronic myelocytic leukemia (CML) patients cultured in diffusion chamber (DC) system. Proc. Soc. Exp. Biol. Med. (in press). 17453

Greenberg, M. L., Chanana, A. D., Cronkite, E. P., Giacomelli, G., Rai, K. R., Schiffer, L. M., Stryckmans, P. A., and Vincent, P. C. The generation time of human leukemic myeloblasts. J. Lab. Invest. 26, No. 3, 245-52 (1972). 17314

Wagner, H. P., Cottier, H., and Cronkite, E. P. Variability of proliferative patterns in acute lymphoid leukemia of children. Blood 39, No. 2, 176-86 (1972). 17226

14. [redacted]:

200 Word Summary:

The overall objective in these studies is understanding of the factors controlling cell proliferation. Specific objectives are: a) to define anomalous cell proliferation in human diseases such as leukemia, auto-immune diseases and malignant lymphomas; b) to better understand the mechanism of allograft reaction and evaluate ECIB and ECIL in the management of allotransplants and in the therapy of human

(See Continuation Sheet)

Project Title: Lymphocytopoiesis and Transplantation Immunology RX-01-03-(d)

14. Scope: (Cont'd)

leukemia; c) to establish the role of bone marrow and thymic-derived lymphocytes in other mammals than the mouse.

The role of the lymphopoietic system in immunity is established. Present doctrine states: antigens are recognized by thymic (T) cells, transformation of bone marrow (B) cells is followed by proliferation and development of clones of B cells producing specific antibody. Factors regulating the proliferation of hemopoietic stem cells (HSC) in the bone marrow and their differentiation into the lymphopoietic system as well as other cell lines are not understood. Through nucleic acid labeling with tritiated precursors, autoradiography, and the detection of specific antigens characteristic of the T lymphocyte, cell proliferation and migration are studied in an endeavor to characterize the kinetic model of lymphocytopoiesis and its regulation.

B) Supplement to 200 Word Summary:

DNA and RNA labeling of lymphocytes by radioactive precursors of nucleic acids is accomplished by: a) intravenous administration to the whole body; b) single organ perfusion *in vivo*; c) *in vitro* incubation; d) *in vitro* and *in vivo* techniques combined; and e) cross circulation of a labeled allogeneic animal or syngeneic twin with an unlabeled partner.

These techniques are applied: a) to estimate cell cycle times of various classes of lymphocytes; b) to study the influence of concentration of lymphocytes in blood and/or lymph on cell cycle times and cell proliferation rates; c) to measure cell production in, and cell migration from, individual lymphoid organs such as bone marrow, lymph nodes, spleen, and thymus; d) to study lymphocytopoiesis in animals deprived of major lymphoid organs; e) to characterize the mechanism by which agents such as heparin and *B. pertussis* cause lymphocytosis; f) to study the recirculation of allogeneic and syngeneic lymphocytes; and g) to measure the life span of migrant cells from organs labeled by perfusion.

The thymus regulates in part the size of the circulating pool of lymphocytes. The influence of thymectomy on the sizes of the various lymphocytic pools is studied utilizing isotopic dilution and the depletion methods of extracorporeal irradiation of the blood (ECIB) and lymph (ECIL). The interrelationship between the thymus and the bone marrow lymphocytes is studied by observing proliferation and antibody production in diffusion chambers containing mixtures of B and T cells. Regional organ perfusion of bone marrow and thymus by radioactive isotopes is used to study *in vivo* the interaction of migrant B and T cells in lymph nodes and spleen. The proliferating and non-proliferating lymphoid cells are tagged with various radioisotopic labels to study their migration patterns, life span,

(See Continuation Sheet)

Project Title: Lymphocytopoiesis and Transplantation Immunology RX-01-03-(d)

14. Scope: (Cont'd)

the changes during depletion and repletion, and the changes in the course of allograft immunization.

Labeling of thymocytes with tritiated thymidine via intra-arterial cannulae which has provided much information about the pattern and magnitude of T cell migration in calves will be used to gain more information on the fate and life span of T cell migrants. The magnitude of migration from the thymus is studied also by observing the natural marker of T cells, the alloantigen θ. The large postnatal migration of mouse θ + T cells to the intestinal lymphoid tissues has been correlated with postnatal population of gut by bacteria, suggesting that the migratory stimulus may be from bacterial antigens. This will be investigated by studying T cell migration into the Peyer's patches and gut lumen using sterile and contaminated exteriorized intestinal loops.

The long term study of tritiated thymidine labeled DNA and cell turnover is complicated by the fact that the tritiated thymidine is reutilized. However, in the lymph node there appears to be either no reutilization or reutilization is at the polynucleotide level thereby escaping destruction by thymidine kinase. A possible method for studying this question is regional lymph node perfusion. Infusion of DNA precursors into the lymph node would label only those cells in DNA synthesis within the lymph node and cells entering the node from other lymphoid organs would not be labeled. Cells leaving the lymph node can be monitored constantly by cannulating the efferent lymphatic duct.

Effective prolongation of kidney allograft survival was obtained with pre- and post-grafting ECIB in some goats. However, for unknown reasons some kidneys were rejected normally. Effective immunosuppression by ECIB may depend upon the degree of histocompatibility between donor and recipient. Methods for determining the "closeness" of match between donors and recipients using the one way mixed leukocyte reaction and leukocyte typing will be applied. For ultimate clinical application it appears that ECIB, ECIL, or both, will have to be used in conjunction with drug immunosuppressive therapy. The effectiveness of ECIB and ECIL would be greatly enhanced if more lymphocytes could be mobilized from tissue and killed in transit through the blood. ECIB has been applied in patients with chronic lymphocytic leukemia (CLL) with beneficial effects. As patients need treatment these studies will continue with the objective of determining effect on longevity, morbidity, and duration of useful life. ECIB has no harmful effects in contrast to chemotherapeutic drugs that produce severe and sometimes fatal thrombopenia, granulocytopenia, anemia, and immunosuppression. An adequate evaluation will require at least 10-15 years and a continual flow of patients.

(See Continuation Sheet)

Project Title: Lymphocytopoiesis and Transplantation Immunology RX-01-03-(d)

14. Scope: (Cont'd.)

When the human renal transplant program commences at Nassau County Medical Center, patients will be randomized into ECIB prepared and non-ECIB prepared groups for evaluation of ECIB as a pre-transplant immunosuppressive procedure. Evaluation will require at least 100 patients in both groups. With the estimated renal transplantation rate of 40-50 per year it will take about 5 years to evaluate ECIB.

The toxicity of B. pertussis precludes its clinical use. It is necessary to resume work on this lymphocytosis-producing agent in order to separate non-toxic lymphocytosis-promoting factor. Another question that remains unanswered is the role of stress-induced secretion of adrenal cortical steroids on induction of lymphocytopenia during ECIB and ECIL. This problem will be studied by performing ECIB and ECIL in normal, adrenalectomized, and sham-adrenalectomized calves.

The culture of cells in intraperitoneal diffusion chambers described in RX-03-02-(b) has provided provocative data on lymphocytic growth. Human blood cells in diffusion chamber cultures show a sequence of events suggesting that small lymphocytes are undergoing transformation and proliferation into lymphocytoid blasts with production of plasma cells after 2-3 weeks in culture. The diffusion chamber system will be applied in the study of immunological reactions.

15. Relationship to Other Projects:

Closely related studies at Brookhaven are the studies of hemopoiesis (RX-03-02-b) and Stoner's studies on lymph nodes during antigenic stimulus (RX-03-01-b). At Oak Ridge Makinodan studies cellular aspects of humoral antibody production during aging, and Hanna studies immunological systems in relation to cancer, with emphasis on cellular immunity.

Cottier, University of Bern; Jansen, South African Atomic Energy Board, Pretoria; Killmann, Copenhagen; Fliedner, University of Ulm; and Schiffer, Allegheny General Hospital, Pittsburgh, all alumni of Brookhaven, continue to work in closely allied areas and in collaboration with BNL.

Related studies elsewhere include those of: Thomas and associates, University of Washington, School of Medicine, on ECIB in leukemia and bone marrow transplantation; and Hollard and associates at Centre d'Etudes Nucleaires de Grenoble, France, on ECIB and its application in the treatment of leukemia. McGinn and associates at the University of Cambridge, Persson and associates at the University of Goteberg, and Weeke and associates, Rigshospitalet, Copenhagen, apply ECIB in the preparation of patients for

(See Continuation Sheet)

Project Title: Lymphocytopoiesis and Transplantation Immunology RX-01-03-(d)

15. Relationship to Other Projects: (Cont'd.)

renal transplantation. Other clinical ECIB studies are those of: Anderson, University of Copenhagen; Lajtha, Manchester, England; and Mauer, Children's Hospital, University of Cincinnati.

Gowans and associates, Sir William Dunn School of Pathology, Oxford; Morris et al. University of CANBERRA, Australia; Lance and associates, Northwick Park Hospital, Harrow, England; Everett and associates, University of Washington, and Waksman and associates at Yale University study cell proliferation and migration in lymphopoietic systems and the relationship to immunologic reactions.

16. Technical Progress in FY 1973:

Intra-arterial labeling of the thymus with tritiated thymidine in calves has shown extensive migration of thymocytes to the gut-associated tissues, lymph nodes, and spleen. Within lymph nodes, the migration first to the paracortex is followed by migration to the outer cortex and into the medulla. Thymic cells are not found in germinal centers nor have any, to date, been found in the bone marrow. In the spleen, labeled cells are first seen in the marginal zone; migration follows into the dense white cortex and into the red pulp of the spleen. Preliminary studies suggest that thymic migrants divide rapidly and are undetectable after 96 hours. The life span of the migrants is under study.

Brookhaven results, based on morphological and autoradiographic studies of thymic cell migration and proliferation in newborn mice, conflicted with data obtained elsewhere utilizing cytotoxicity of antibodies against the thymic alloantigen θ. While at the University of Bern, Dr. Chanana restudied the problem employing indirect immunofluorescent techniques directed against natural θ surface alloantigen. These studies conclusively demonstrated a major migration from thymus to the gut-associated lymphoid tissue, lymph nodes, and spleen in the first five postnatal days. The magnitude of the migration of θ x cells from the thymus of the mouse to gut was unexpected--over 90% of the cells in Peyer's patches and mesenteric lymph nodes of 4-day old mice were θ positive. These results fully support earlier studies in calves on migration from thymus to peripheral tissues using tritiated thymidine and thymic specific antigen as markers; also the FY 1972 studies of Joel on migration of tritiated thymidine labeled cells from the thymus of the postnatal mouse. The assertion that the gut associated lymphoid tissue is primarily a bone marrow dependent organ must therefore be in doubt.

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

Routes of migration of lymphocytes from the calf thymus were shown to be through the thymic lymphatics and veins. The daily output of lymphocytes from the thymus is 3-4 times greater than the number of small lymphocytes in the blood.

The question of magnitude of thymic cell migration to bovine Peyer's patches is unresolved. The animal studies were completed, but the autoradiographic data analysis is not. Most investigators studying thymic lymphocytopoiesis and migration either label suspensions of thymic cells or inject directly into the thymus, distorting its architecture. Since studies following intra-arterial perfusion of the thymus with tritiated thymidine showed a different distribution than the above techniques, distribution of thymic cells after intravenous injection of labeled thymic cell suspension was compared to that following intra-arterial perfusion. Following i.v. injection a greatly increased trapping of thymic cells within the spleen was observed, suggesting that infusion of thymic cell suspensions may provide quantitatively erroneous results.

Since transplanted allogeneic lymphocytes are believed responsible for the development of graft vs host disease after bone marrow transplantation, the capability of isogeneic and allogeneic lymphocytes to recirculate from blood to lymph was investigated using in vivo and in vitro thymidine and cytidine labeling followed by transfusion or cross circulation. Preliminary results show that isogeneic lymphocytes are capable of recirculating from blood to lymph whereas the allogeneic are not endowed with this capacity. This work was planned for completion in FY 1973 but due to lack of personnel its completion is now planned for FY 1974.

The turnover, in various lymphoid organs, of DNA labeled with tritiated thymidine was compared to that of DNA labeled with I-125-deoxyuridine using mice 1-8 days after birth. Significant differences in the exponential declines of these two isotopes indicate that in addition to immigration, there is a significant cell death taking place in the thymus during this period of development. Similar analyses of peripheral lymph nodes suggest that cell death and reutilization of thymidine occurs in these organs. This is contrary to findings in lymph nodes of young adult mice and further studies are underway utilizing isolated lymph node perfusion to eliminate the effect of migration of labeled cells into and out of the lymph node.

The response of the lymphocytic system of the goats to ECIB was studied during and up to two months after ECIB by observing changes in the lymphocyte concentration and the "flash" labeling index of lymphocytes in blood, thoracic duct lymph, thymus, prescapular lymph node, spleen,

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd)

Peyer's patch, and bone marrow. Lymphocyte concentration in the blood declined continuously during ECIB. Repletion of lymphocytes began a few days after cessation of ECIB and continued for the duration of the study. Complete recovery, however, was not achieved by 2 months after termination of ECIB. Changes similar to those seen in blood lymphocyte concentration (decrease and recovery) were also observed in the thoracic duct lymph. The spleen, lymph nodes, thymus, and Peyer's patches showed various degrees of lymphocyte depletion during ECIB. Lymphocyte concentration in the bone marrow remained constant. Within the two-month experimental period, the thymus, lymph nodes and Peyer's patches recovered completely while the spleen, despite showing some recovery, remained partially depleted for the entire period. Following cessation of ECIB, the lymphocyte concentration in bone marrow increased, reaching a level above the controls by day 4. Thereafter it returned to the level of the mean sham control groups and appeared to remain below this level for the entire period of the study. During ECIB an increase in the flash labeling index of the dividing population of lymphocytes was noticed in the bone marrow, Peyer's patches, blood and thoracic duct lymph. The increase was most marked in the bone marrow. The peak level of the labeling index of the bone marrow was attained on day 4 following cessation of ECIB, the time at which the lymphocyte concentration of bone marrow also peaked. Thymus and spleen did not show any appreciable change in the labeling index as a result of ECIB. An increase in labeling index of the dividing population of lymph node lymphocytes was observed during ECIB and sham ECIB. The observation that ECIB-induced lymphocytopenia initiated a stimulatory signal for increased proliferative activity of lymphocytes in the bone marrow is new and exciting and may shed light on one of the original objectives of ECIB, namely, and to show whether partial depletion of lymphocytic tissues triggers proliferation within some segment of the lymphocytic system. Whether this increased proliferation is in response to increased cell destruction, or to a mechanism that senses a decrease in size of lymphocyte pools in blood or tissues is not known. Earlier studies had shown that peripheral lymphocyte depletion results in an initial, temporary depletion in the thymus during ECIB. The thymus is known to be repopulated by cells originating in the bone marrow after fatal irradiation. These facts, and the increased proliferative activity observed in bone marrow, suggest that there might be a feedback loop from the thymus to the marrow, stimulating marrow production to replete the thymus.

During the preceding studies, the lymphocyte count/unit volume of thoracic duct lymph continued to fall, reaching the lowest level a few days after cessation of ECIB. To study this further, thoracic ducts were cannulated and arteriovenous shunts established in calves. One calf was subjected to intermittent ECIB and the other received intermittent sham

(See Continuation Sheet)

Project Title: Lymphocytopoiesis and Transplantation Immunology RX-01-03-(d)

16. Technical Progress in FY 1973: (Cont'd)

ECIB. Thoracic duct cells, labeled in vitro with tritiated thymidine to tag a fraction of the large cells, were infused intravenously. The total output of labeled and unlabeled lymphocytes in thoracic duct lymph and the concentration of labeled and unlabeled lymphocytes in the blood was monitored daily, during and following ECIB. The total output of labeled and unlabeled lymphocytes in thoracic duct lymph declined. Blood lymphocyte concentration showed similar changes. Cell size analysis in thoracic duct lymphocytes showed that small non-dividing lymphocytes and large dividing lymphocytes were decreased.

To reduce costs to meet budgetary restrictions, a project on the influence of long term thymectomy on lymphocytopoiesis and transplantation immunology was terminated by killing 30 goats that had been followed for up to five years after thymectomy. This project will not be re-activated until it is certain that adequate manpower will be available on a long-term basis.

Three schedules of ECIB have been used to prepare goats for renal transplants. Randomly selected donors and recipients have been used. With the exception of ECIB-I, donor-recipient pairs checked for "closeness of match" with a one-way lymphocyte culture technique. ECIB-I consisted of irradiating 5-10 blood volumes per day for 10-15 days prior to transplantation with a high transit dose varying from 275-400 rads. This schedule had provided encouraging results when used as an adjunct with standard immunosuppression in preparing human beings for renal transplantation at the Rigshospitalet University of Copenhagen, Denmark, and at the Department of Surgery, University of Goteberg, Sweden. ECIB-I was used without any supportive chemical immunosuppressive therapy or donor-recipient matching. The results were disappointing and hemolysis was induced. ECIB-II shcedule was devised to reduce dose to red cells and time required for pre-transplant perparation. Fifteen to eighty blood volumes were irradiated each day for three to eight pre-transplant days, the transit dose ranging from 35-50 rads. Some animals retained kidney grafts for significantly longer periods. There was, in general, a good correlation between closeness of match obtained from the one way leukocyte culture and the length of allograft survival in the control and the sham-ECIB groups. In the group receiving ECIB, however, the longest survivor was very poorly matched with its donor. These limited results suggest that the degree of histocompatibility may not be the only factor determining the success of immunosuppression with ECIB. The results of renal transplants are: (1) untreated recipients, 12 transplants 19 days mean survival (range 15-35 days); (2) ECIB-I, 8 transplants, 18 day mean survival (range 14-24 days); (3) ECIB-II, 9 transplants, 30 day mean survival (range 2-58 days); (4) ECIB-III, 13 transplants, 48 day mean survival (range 19-84 days); (5) Sham ECIB,

(See Continuation Sheet)

Project Title: Lymphocytopoiesis and Transplantation Immunology RX-01-03-(d)

16. Technical Progress in FY 1973: (Cont'd)

10 transplants, 25 day mean survival (range 13-34 days).

In October 1972 the renal transplants were temporarily discontinued due to budgetary restrictions.

17. Expected Results in FY 1974:

Analysis of autoradiographs in thymocyte migration, life span and lymphocyte recirculation will be completed, data analyzed and submitted for publication. Size distribution data collected over 7 years on lymphocytes during ECIB will be analyzed. Studies on role of the adrenal in ECIB-L induced lymphopenia will commence using adrenalectomized calves maintained on level steroid intake.

Thymic cell migration studies will continue with efforts to determine the life span of thymic migrants in the peripheral lymphoid tissues and whether the lymphocytes known to migrate into the lumen of the gut are B or T cells. If the apparent stimulatory influence of lymphopenia upon bone marrow lymphopoiesis is confirmed, this apparent breakthrough in the study of lymphopoiesis will be exploited with studies focused on the possible thymic bone marrow feedback loop.

Lymphoid cell mixtures will be grown in diffusion chambers for evaluation of the role of T cells in antigeneic recognition, their interaction with B cells, and the identity of cells that produce antibodies.

Studies on ECIB in renal transplantation in goats will be reactivated if the Large Animal Facility can be operated seven days a week. Studies will include: combination of ECIB and ECIL to further elucidate the mechanism of allograft rejection and to further prolong the survival of renal transplants; combination of ECIB and immunosuppressive chemicals to determine the efficiency of these procedures for eventual application in human patients; "matching" of recipient-donor pairs in combination with mixed breeding; and in vitro studies of the influence of "killer" cells on kidney cells.

Studies on the regional perfusion of various lymphoid organs with radioactive isotopes have been limited by budgetary problems. These studies are designed to enable better understanding of the patterns and magnitude of lymphocyte traffic under more or less physiologic conditions. This kind of information is essential to understand the overall phenomenon of lymphocytopoiesis. Thymus perfusion studies will be extended to bone marrow, spleen and lymph nodes. After present data are analyzed, regional perfusion of lymphoid organs with tritiated thymidine will be undertaken with emphasis on bone marrow perfusion.

(See Continuation Sheet)

17. Expected Results in FY 1974: (Cont'd)

A study combining θ allo-antigen and tritiated thymidine as markers to follow thymic cell migrants and their peripheral fate will begin in late FY 1974.

ECIB treatment of chronic lymphocytic leukemia will be reactivated as patients under study in out-patient department require therapy.

If Nassau County Medical Center program on renal transplantation commences, BNL will apply ECIB pre-treatment to patients randomly selected by age, sex and disease state.

18. Expected Results in FY 1975:

All studies reported for the previous year will continue with directions determined by the new findings.

If and when a biochemist can be afforded, studies on separation of the lymphocytosis factor from B. pertussis will be once again undertaken to search for an agent that may potentiate ECIB in preparing animals for kidney transplantation and patients for treatment of chronic lymphocytic leukemia.

It is hoped to reactivate a long dormant study on ECIB therapy of acute myelocytic leukemia evaluating the effect of transit dose on survival time and morbidity.

Thymic cell migration studies should be phased out during this year and studies on immunity in diffusion chambers intensified.

19. Description and Explanation of Major Materials, Equipment and Subcontract Items:

FY 1975 Capital Equipment:

The major capital acquisition is an automated autoradiographic grain counter and cyto-analyzer which would replace the traditional visual counting proce[redacted] now laboriously accomplished by scientific and technical personnel at a [redacted] which drastically limits the rate of data acquisition and analyses of pr[redacted]eration systems and thus the number of patients that can be studied. As the equipment will be used in the research reported in four separate budget activities the cost is prorated $25,000 each to RX-01-01-a, RX-01-03-d, RX-03-01-b and RX-03-02-b. Another important acquisition for the hemopoietic studies is a blood cell separator to improve the separation of cells for

(See Continuation Sheet)

Project Title: Lymphocytopoiesis and Transplantation Immunology RX-01-03-(d)

19. Description and Explanation of Major Materials, Equipment and Subcontract Items: (Cont'd.)

FY 1975 Capital Equipment: (Cont'd.)

transplantations of the hematopoietic stem cells. Used principally in two studies $20,000 each is prorated to RX-01-03-d and RX-03-02-b.

20. Proposed Obligations for Related Construction Projects:

None

(See Continuation Sheet)

Project Title: Nuclear Medicine Technology and Other Health Applications
Lymphocytopoiesis and Transplantation Immunology RX-01-03-(d)

Reiteration of Assurance Statement
on
Investigation Involving Human Subjects
Including Clinical Research

The Hospital of the Medical Research Center, Brookhaven National Laboratory, complies with the policy for the protection of human subjects participating in activities supported directly or indirectly by grants or contracts from the Department of Health, Education, and Welfare and from the Atomic Energy Commission. In fulfillment of its assurance, this institution has established a standing committee to review investigations involving human subjects, including clinical research, and said committee is charged with responsibilities to determine for each proposed study that:

the rights and welfare of each patient will be adequately protected,
that the potential benefits outweigh the risks, and
that an informed consent will be obtained from each patient.

The committee, appointed by the Director, Brookhaven National Laboratory, provides for independent judgment of its members, and alternates are provided to serve when there may be conflict of interest. This institution encourages constructive communication between the committee and principal investigators on the safeguarding of the rights and welfare of subjects; will provide the required care for persons injured as a result of participation; and maintains complete documentation of proposals and reviews. Approved investigations are reviewed by the committee at least annually and the institution periodically reviews its practices and procedures to assure they are consistent with the policies published by the Department of Health, Education, and Welfare.

As of March 7, 1973, the Institutional Review Committee for review of Medical Department research activities involving human subjects is composed of:

J. S. Robertson, M.D., Chairman
G. C. Cotzias, M.D., Alt. Chairman
S. H. Cohn
E. A. Popenoe, Alt. for Dr. Cohn
G. Chikkappa, M.D.
H. Connell
R. A. Love, M.D.
G. Price
N. P. Rathvon, Jr.
D. N. Slatkin, M.D., Alternate
A. P. Wolf, Alternate
L. K. Dahl, M.D. - Clinical Consultant
W. A. Finn - Administrative Consultant
C. W. Flood - Health Physics Consultant

Signature: Eugene P Cronkite

Title: Chairman, Medical Department

Date: March 7, 1973

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory — RX-Biomedical & Environmental Research
Laboratory — Program

1. Contractor: Associated Universities, Inc. — Contract No.: AT(30-1)-16 — Task No.:

2. Project Title: Nuclear Medicine Technology and Other Health Applications
Metabolism of Carbon Labeled Compounds — 189 No.: RX-79

3. Budget Activity No.: RX-01-03-(e)

4. Date Prepared: May 1973

5. Method of Reporting:
Scientific Meetings
Monthly Letter to AEC
Scientific Journals

6. Working Location:
Brookhaven National Laboratory

7. Person in Charge:
L. V. Hankes
W. W. Shreeve

Principal Investigator:
L. V. Hankes
W. W. Shreeve

8. Project Term:
Continuing
From: To:

9. Man-Years:

Direct Man-Years	FY 1973	FY 1974	FY 1975
Scientific & Professional	1.0	1.0	2.0
Other	6.0	2.0	1.0
Guests & Res. Collaborators	1.5	---	---
Total	8.5	3.0	3.0

10. Costs (In Thousands of Dollars):

	FY 1973	FY 1974	FY 1975
Research Division	74	78	104
Hospital Division	107	7	0
Research Costs	181	85	104
Equipment Obligations	0	5	5

11. Reactor Concept:

12. Materials:

13. Publications:

Hoshi, M. and Shreeve, W.W. Release and production of insulin by isolated, perfused rat pancreatic islets. Diabetes. 22, No. 1, 16-24 (1973).

14. Scope:

A) 200 Word Summary:

A long range goal in this activity has been a better understanding of the pathogenesis of diabetes and other metabolic diseases. Carbon labeled intermediates of carbohydrates and lipids were used to study the dynamics of interconversion and turnover of carbohydrates and lipids. The effect of hormonal and nutritional variations also was investigated. A corollary has been the development of isotopic tracers of diagnostic value which may be useful for guidance of therapy with drugs, special diets, hormones or vitamins. Extensive studies have been carried out using sugars labeled with carbon-14. More recently, carbon-13 and carbon-11 labeling has offered challenging opportunities.

The amino acid, tryptophan, produces ortho-amino-phenol type compounds believed to induce cancer in animal urinary bladders. In several types of cancer and in other diseases of man, the literature reports significant increases in urinary levels of some tryptophan metabolites. Carbon-14 labeled metabolites are used in animals and man to determine the normal tryptophan metabolic pathway. The labeled metabolites include anthranilic acid, kynurenine, hydroxykynurenine, and hydroxyanthranilic acid. Diseases studied include anemias, scleroderma, siderosis, malaria, pellagra, and various cancers.

B) Supplement to 200 Word Summary:

The relative conversions *in vivo* of intermediate carbohydrates (e.g., lactate, pyruvate, glycerol, and malate) to blood glucose, liver glycogen and carbon dioxide in diabetic states in man and animals have been previously studied in this laboratory with carbon-14 labeled precursors. So also has the oxidation of glucose-C-14 to $^{14}CO_2$ been investigated. Similar studies are now done or planned with sugars labeled with carbon-13 (the nonradioactive, stable isotope) with emphasis on diagnostic application. Carbon-13 will be generally preferred for this purpose because there will be no radiation exposure as with C-14.

[redacted] current studies of oxidation of galactose-U-C-14 (and eventually galactose-U-C-13) to CO_2 are similarly directed toward the diagnosis of hypothyroidism, of liver disease and possibly of diabetes, since in these conditions, intolerance for galactose has either been demonstrated or can be expected on theoretical grounds. A highly valuable, potential aspect of the use of galactose-U-C-13 is the oppor-

(See Continuation Sheet)

14. Scope: (Cont'd)

tunity to investigate formation, turnover, and (uniquely possible with C-13) even chemical structure of complex carbohydrates in serum glycoproteins, most of which contain galactose.

Other studies with labeled sugars are concerned with lipogenesis in the liver and with plasma lipid formation and removal. The differences between starch and sucrose in their lipogenic and insulinogenic potential are presently under investigation with human subjects. These studies (with sucrose-C-14, glycerol-H-3, and occasionally C-11-labeled sugars) help to clarify mechanisms of susceptibility to hyperlipemia and hyperinsulinism as affected by different diet (e.g., low or high sucrose) or by steroids. Possibly they can identify particular susceptibilities in individual patients. In this case, C-13 may be valuable diagnostically in this area.

Because of known or suspected changes in diabetes and in steroid-treated subjects, the metabolism of tryptophan-C-13 (or of tryptophan-C-14) to $^{13}CO_2$ (or $^{14}CO_2$) and to intermediate metabolites in accessible body fluids will be examined in such patients. This unexplored direction may provide new diagnostic parameters in certain metabolic diseases, in vitamin deficiencies, e.g., pellagra, and in distrubances of the central nervous system. (Shreeve)

The following work was funded for FY 1973 and reported in RX-01-03(c) (old code 06-03-01-c) but is reported here also for convenient reference in relation to work in future years.

The objective of the project studying tryptophan metabolism in normal animals and man and in various diseases using tryptophan metabolites labeled in different positions of their structure with carbon-14 is to see if there is any cause-effect relationship in the diseases studied. The literature reports show that in several types of cancer patients and patients with other diseases a significant increase occurred in the levels of acetylkynurenine, kynurenine, kynurenic acid, hydroxykynurenine, xanthurenic acid, and quinolinic acid.

The tryptophan metabolites, anthranilic acid carboxyl-C-14, kynurenine-keto-C-14, kynurenine-ring-2-C-14, hydroxykynurenine-keto-C-14, and [redacted]oxyanthranilic acid-carboxyl-C-14 have been synthesized. Procedures were [redacted]ected which utilized columns filled with paper pulp for the reso[redacted]on of small quantities of the L- and D-isomers of tryptophan, kynurenine, and hydroxykynurenine. Size and resolving power of the columns limit the quantity of material which may be resolved (50-300 mg) and meet FDA specifications. These factors in turn limit the number and the rate at which studies may be done. The metabolism of carbon-14 labeled

(See Continuation Sheet)

14. Scope: (Cont'd)

metabolites was first studied using the same body weight and dose levels that would be used in humans, and has now been extended to humans. The results obtained with the isotopically labeled compounds should help determine the specific abnormalities in the tryptophan metabolism in patients and eventually produce information that may lead to better therapy. Patients with scleroderma, siderosis, pellagra, and hepatic tumors are studied in South Africa. Malaria is studied in Stateville Prison, Illinois, and in South Africa. The labeled compound isolation and analytical work is done at Brookhaven, though in the past, some of this work was accomplished at the University of Wisconsin. (Hankes)

15. Relationship to Other Projects:

Studies related to the work on carbohydrate and lipid metabolism include those at Brookhaven of Steele, Department of Biology; Dahl, Medical Research Center (see RX-01-03-b); Atkins (see RX-01-03-c); Wolf and Christman, Department of Chemistry; and Newman, Department of Applied Science. At Los Alamos Scientific Laboratory, related work includes the collaborative studies with Ott and Gregg on production of C-13 labeled organic compounds for metabolic studies; MacInteer on analyses of $^{13}CO_2$; and Matwiyoff on NMR analyses of glucose-C-13. Klein of Argonne National Laboratories collaborates on analysis of C-13 labeled compounds.

Related studies at other institutions include those of Waterhouse, University of Rochester; Searle, V.A. Hospital, San Franciso; Kreisberg, University of Alabama; Pollycove, San Francisco General Hospital, University of California; Da Costa, Radiation Medicine Centre, Bombay, India; Segal, Pennsylvania Medical College; Shoop, University of New Mexico; Hetenyi, University of Toronto; Kalant, University of Montreal; Long, Columbia University; Farquhar, Stanford University; Bierman, University of Washington; Nikkila, University of Helsinki; Sailer, Universitatsklinik in Innsbruck; MacDonald, Guy's Hospital Medical School, London; Nestel, Australian National University, Canberra; Chytil, Vanderbilt University and Curzon, Institute of Neurology, London. (Shreeve)

Generally, tryptophan metabolites labeled with carbon 14 in positions which originate from specific positions of the benzene ring or the indole nucleus are unavailable due to difficulties encountered in their synthesis, and separation of their D- and L-isomers in satisfactory yields. Although studies with carbon-14 labeled isomers of this type of compound are conducted only at Brookhaven, studies in other laboratories include those of: Altman, N.Y. Medical College, on hormone effects on tryptophan pyrrolase enzyme; Gholson, Oklahoma University, on feedback control mechanisms; Lardy, University of Wisconsin, on

(See Continuation Sheet)

15. Relationship to Other Projects: (Cont'd)

tryptophan metabolite inhibition of glycolysis; Miller, University of Rochester, on induction of the tryptophan pyrrolase enzyme; Wagner, Vanderbilt University, on tryptophan metabolite inhibition; and Hayaishi, Kyoto University, Japan, on pyridine nucleotide synthesis in animals. Some of these investigators suggest that the stimulation or depression of the pyrrolase enzyme is the factor that controls the abnormalities seen in urinary tryptophan metabolite excretion. Studies at BNL gave evidence that other factors such as vitamins and diet are also involved. (Hankes)

16. Technical Progress in FY 1973:

Clinical studies with C-13 labeled sugars were commenced during the past year with the use of glucose-U-C-13 in a test of oxidation to $^{13}CO_2$ (in the breath) after oral administration in a glucose load. Studies were begun with glucose-U-C-13 instead of glucose-1-C-13, as intended a year ago, because of more rapidly successful biosynthesis of glucose-U-C-13 in adequate quantities. Seven non-diabetic and six diabetic subjects (distributed between the University of New Mexico and Brookhaven National Laboratory in a joint study) were tested to date. One diabetic with excessive glucose intolerance oxidized glucose-U-C-13 at less than half the mean normal rate, but all other diabetics, who had mild glucose intolerance (according to blood glucose concentration), were within the normal range of oxidation. This suggests that this test will not be generally useful for diagnosis of early or sub-clinical diabetes. However, the findings imply that continued high rates of hepatic gluconeogenesis after a glucose load account largely for glucose intolerance in mild diabetics. Therefore, studies were initiated to measure plasma glucose-C-14 after oral administration of L-lactate-U-C-14 in load quantity; the formation of $^{14}CO_2$ is also observed, since earlier studies indicated that formation of $^{14}CO_2$ is diminished as well as formation of glucose-C-14 increased from lactate-C-14 (intravenously) in diabetics.

The study of interacting effects of high sucrose diet and of anovulatory steroids on formation and turnover of plasma triglycerides was continued by the inclusion of eight more obese women. Effects of these parameters on conversion of ingested sucrose-C-14 to triglycerides, turnover of triglycerides after labeling with glycerol-2-H-3, insulin response to sucrose load, and plasma post-heparin lipolytic activity (PHLA) were studied. Results confirm previous findings that more sucrose is converted to triglycerides after high-sucrose than after high-starch diet and during regimens of estrogen-containing contraceptives, but not those with only progestagens. PHLA of plasma (after glucose) is diminished on high-sucrose diets, but turnover of plasma triglycerides in the post-absorptive state is not significantly changed. This study was principally supported by non-AEC funds.

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd)

Although a few more patients were included this year in the collaborative studies of oxidation of galactose-U-C-14 to breath $^{14}CO_2$ in hypothyroid patients and in patients with liver disease (initiated the previous year) at the Radiation Medicine Centre in Bombay, no definite results are available. The biosynthesis of galactose-U-C-13 (intended for the same application) was pursued at Los Alamos Scientific Laboratory but encountered technical difficulties, so the compound is not yet available. However, tentative arrangements were made for its use both at the University of New Mexico and at Brookhaven National Laboratory.

Chemical synthesis of lactose-1-C-13 was initiated at Los Alamos Laboratory. This compound will be used in an oxidation test for lactose intolerance in patients at University of New Mexico hospitals. (Shreeve)

A metabolic study of the L-isomers of carbon-14 labeled tryptophan, kynurenine, and hydroxykynurenine in patients with scleroderma was conducted at the F.W. Verwoord Hospital in Pretoria, South Africa. The purpose of the study was to determine whether the South African type of scleroderma had metabolic abnormalities similar to those of the American type. The South African scleroderma patients were male caucasian miners whose illness began with silicosis followed by later development of scleroderma. Breath carbon dioxide samples were collected in sodium hydroxide over a 24-hour period after the C-14 labeled doses were given and 24-hour urine samples were collected for a three day period. The breath $^{14}CO_2$ level for those given tryptophan-7a-C-14 was 6.3 - 8.9 percent; L-hydroxykynurenine-keto-C-14 was 30.6 - 37 percent and L-kynurenine-keto-C-14 was 48.6 - 63.3 percent. The values are similar to those found in the American scleroderma patients given the same compounds. Kynurenine, hydroxykynurenine, xanthurenic acid, kynurenic acid, picolinic acid, quinolinic acid, N-methylnicotinamide, N-methyl-2-pyridone-5-carboxamide and nicotinic acid will be isolated from the urine by carrier techniques and the carbon 14 content of these urinary components determined. The results from these analyses should help determine the particular metabolic abnormalities in males with this disease. This long range project has made little progress due to insufficient technical assistance. The South African Atomic Energy Board (SAAEB) is sending Dr. E.J.P. de Bruin to Brookhaven to spend a year of post-doctorate study at their expense and to help with the collaborative projects. Dr. de Bruin will work on the scleroderma, pellagra, and hepatic tumor problems.

The tryptophan metabolic studies in prisoners with a sensitivity to primaquin drugs in the treatment of malaria, were expanded to include a study of kynurenine metabolism. In collaboration with Dr. Carson at Stateville Prison, Illinois, urine samples were collected from five prisoners before and after loading with tryptophan. The urines were analyzed for 16 tryptophan metabolites to provide control values for other

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd)

studies of the American Negro and African Bantu. Some subjects were given 400 mg doses of carbon-14 labeled L-kynurenine and the study requires several more subjects receiving 200 mg doses.

Observations reported last year suggest that South African pellagrins have subnormal vitamin B_6 coenzyme levels in addition to the previously proposed stress-induced increase in the activity of tryptophan pyrrolase or a lack of feedback control of this enzyme by low pyridine nucleotides. A grant (strongly recommended by the Japanese-American Study Commission) has been obtained from the DHEW to provide two technicians to continue this internationally important project.

In 1972 a limited number of pellagra patients were studied during the pellagra season in SouthAfrica. At the request of Dr. Roux, President, and Dr. Jansen, Head of the Life Sciences Division, SAAEB, the study will be completed in 1973 with travel and laboratory expenses paid by SAAEB. In addition, patients with primary hepatoma will also be studied. This hepatoma is nutritionally based, causes death within three months, and has a mortality rate of more than 150 per hundred thousand per year among the Bantu.

A BNL research collaborator, Dr. Ludwig Feinendegen, Director of The Institut for Medicine, KFA, Julich, Germany, has requested a week's visit by the Principal Investigator to set up a collaborative study at Julich utilizing double labeled tryptophan. (Hankes)

17. Expected Results in FY 1974:

Due to the resignation of the Principal Investigator on carbohydrate and lipid metabolism to accept a position as Head, Nuclear Medicine, Veterans Administration Hospital, Northport, New York, this area of the medical research program will be phased out. During the fiscal year, there will be continuing expenses in order to complete the clinical responsibilities to patients participating in the study. (Shreeve)

Work on the mechanism of formation of the 8-methyl ether of xanthurenic acid in humans is planned. Before conclusive statements can be made concerning mechanisms, studies of enzyme methylation reactions forming methoxykynurenine and methoxyanthranilic acid are required. Difficulties in obtaining fresh human autopsy material may delay completion of this project. The relationship of these xanthurenic acid compounds to insulin and its control of carbohydrates will be investigated by Dr. de Bruin.

Additional compounds will be prepared, resolved and standardized for further studies of pellagra, scurvy and hepatic cancer patients in South Africa and malaria patients at Stateville Prison in Illinois.

(See Continuation Sheet)

17. Expected Results in FY 1974: (Cont'd.)

The study of carbon-14 labeled tryptophan, kynurenine, and glucose in malaria patients will be continued as material becomes available and FDA approval is obtained.

Blood and urine samples from the pellagra and scurvy studies will be analyzed for all of the individual amino acids, as technical help becomes available and is trained. Work will continue on blood and urine samples from hemosiderotic (scurvy) patients who had been given labeled ascorbic acid in South Africa. (Hankes)

18. Expected Results in FY 1975:

The study of tryptophan metabolism in scleroderma, scurvy, pellagra, malaria, anemias, hepatic cancer and other related diseases will be continued. An additional trip to South Africa is anticipated at the invitation and expense of the South African Atomic Energy Board to carry out additional studies.

19. Description and Explanation of Major Materials, Equipment, and Subcontract Items:

None

20. Proposed Obligations for Related Construction Projects:

None

(See Continuation Sheet)

Nuclear Medicine Technology and Other Health Applications

Project Title: Metabolism of Carbon Labeled Compounds RX-01-03-(e)

Reiteration of Assurance Statement
on
Investigation Involving Human Subjects
Including Clinical Research

The Hospital of the Medical Research Center, Brookhaven National Laboratory, complies with the policy for the protection of human subjects participating in activities supported directly or indirectly by grants or contracts from the Department of Health, Education, and Welfare and from the Atomic Energy Commission. In fulfillment of its assurance, this institution has established a standing committee to review investigations involving human subjects, including clinical research, and said committee is charged with responsibilities to determine for each proposed study that:

the rights and welfare of each patient will be adequately protected,
that the potential benefits outweigh the risks, and
that an informed consent will be obtained from each patient.

The committee, appointed by the Director, Brookhaven National Laboratory, provides for independent judgment of its members, and alternates are provided to serve when there may be conflict of interest. This institution encourages constructive communication between the committee and principal investigators on the safeguarding of the rights and welfare of subjects; will provide the required care for persons injured as a result of participation; and maintains complete documentation of proposals and reviews. Approved investigations are reviewed by the committee at least annually and the institution periodically reviews its practices and procedures to assure they are consistent with the policies published by the Department of Health, Education, and Welfare.

As of March 7, 1973, the Institutional Review Committee for review of Medical Department research activities involving human subjects is composed of:

J. S. Robertson, M.D., Chairman
G. C. Cotzias, M.D., Alt. Chairman
S. H. Cohn
E. A. Popenoe, Alt. for Dr. Cohn
G. Chikkappa, M.D.
H. Connell
R. A. Love, M.D.
G. Price
N. P. Rathvon, Jr.
D. N. Slatkin, M.D., Alternate
A. P. Wolf, Alternate
L. K. Dahl, M.D. - Clinical Consultant
W. A. Finn - Administrative Consultant
C. W. Flood - Health Physics Consultant

Signature: Eugene P Cronkite

Title: Chairman, Medical Department

Date: March 7, 1973

RX-87

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory
Laboratory

RX-Biomedical & Environmental Research
Program

1. Contractor:
Associated Universities, Inc.

Contract No.: AT(30-1)-16

Task No.:

2. Project Title:
Nuclear Medicine Technology and Other Health Applications
Early Detection and Localization of Pulmonary Impairment

189 No.:

3. Budget Activity No.:
RX-01-03-(f)

4. Date Prepared:
May 1973

5. Method of Reporting:
Bimonthly Progress Report
Publication in Open Literature
Scientific Meetings
Final Topical Report

6. Working Location:
Brookhaven National Laboratory

7. Person in Charge:
E. P. Cronkite
W. E. Winsche
L. G. Stang, Jr.

Principal Investigator:
H. L. Atkins
P. Richards
H. Susskind

8. Project Term:
From: To:
To be initiated in FY 1975

9. Man-Years:

Direct Man-Years	FY 1973	FY 1974	FY 1975	FY
Scientific & Professional	—	—	1.5	2.5
Other	—	—	3.0	3.0
Guests & Res. Collaborators	—	—		
Total	—	—	4.5	5.5

10. Costs (In [redacted]sands of Dollars):

	FY 1973	FY 1974	FY 1975	FY 197
Research Costs	—	—	150	
Equipment Obligations	—	—	25	

11. Reactor Concept:

12. Materials:

1179246

Nuclear Medicine Technology and
Other Health Applications
Early Detection and Localization of
Project Title: Pulmonary Impairment RX-01-03-(f)

13. Publications:

This proposal initiates new work. However, the feasibility of the proposed technique was presented in prior publications:

Susskind, H., Richards, P., Atkins, H. L., and Skolnick, M. Detection of impaired pulmonary function with a mass-spectrographic tracer technique. Proc. 24th Ann. Conf. Eng. Med. Biol. 13, 228 (1971).

Susskind, H., Richards, P., and Atkins, H. L. Studies of airway closure with helium. J. Appl. Physiol. (Submitted).

Susskind, H., Richards, P., and Atkins, H. L. Closing volume. Ltr. to the Editor, The Lancet, Vol. I. pp. 603-4, 1973.

14. Scope:

(a) 200 Word Summary: The objective is to develop the methodology and instrumentation for two related isotopic tracer techniques, one for the early detection of chronic obstructive pulmonary disease, and the second for the evaluation of the extent and distribution of disease in the lungs. The first technique involves the use of stable helium to measure distal airway closure. Reference values will be prepared from clinical data obtained from a large statistical sample of healthy subjects. Premature closure in subjects indicates abnormalities. The technique is particularly well-suited to mass-screening applications by using mobile equipment and instantaneous computer analysis in the associated "lungmobile". Helium has a number of advantages over other gases, including a higher diffusion rate and a much lower blood solubility.

The second technique involves the use of radioactive xenon 127 in conjunction with the isotope camera and computer interface in clinical studies to quantitate the extent of ventilation and perfusion defects in those patients detected by the helium screening procedure and in others. Xenon 127 has several advantages over xenon 133, the gas currently in use, including higher resolution and more efficient detection of the gammas emitted and a longer half-life (36 days).

(b) Supplement to 200 Word Summary: Emphysema and other diseases of the small airways of the lung, and lung impairment caused by air pollution and tobacco smoke often defy detection in their early stages when treatment could be effective. By the time chronic airway obstruction is detected by the usual measurements of lung function, the disease process is generally irreversible. At the present time, no techniques are available to detect the early stages of chronic obstructive pulmonary disease. Only the airway closure technique and measurement of the maximum mid-expiratory flow rate, which is not as sensitive, show any promise when used in routine pulmonary function tests

(See Continuation Sheet)

1179247

14. Scope: (Cont'd)

or for widespread screening of people.

The benefits to be realized from development of this program result from a reduction in the progression of pulmonary disease by means of early detection and accurate diagnosis. These benefits appear in three areas: (1) reduction in the death rate from chronic obstructive pulmonary disease, estimated to cause 30,000 deaths per year in the U.S. and being a contributing cause to 50,000 others, (2) elimination or at least reduction of the length of hospitalization of pulmonary patients, and (3) reduction of the extent of disability and, hence, of Social Security pension payments for chronic disability costing several hundred million dollars annually.

15. Relationship to Other Projects:

Both techniques will be developed and used in a joint collaborative effort between the Medical and Applied Science Departments at BNL and the hospitals associated with two units of the State University of New York -- the Downstate Medical Center in Brooklyn, under the guidance of Drs. H. S. Lyons and N. A. Solomon, and the Health Sciences Center at Stony Brook, under the guidance of Dr. I. Rezak. The development work, including initial clinical testing, will be carried out at BNL, followed by extensive clinical studies at the well-established pulmonary centers associated with the University.

The airway closure technique is also under development at several laboratories, including McGill University, whose researchers have just recently begun to use inert argon in addition to radioactive xenon 133, Massachusetts General Hospital, where nitrogen 13 is used, and Harvard School of Public Health, where some work with helium is underway. Helium should provide greater sensitivity than other gases, since its high diffusion rate results in the most rapid distribution in all airways, and its lower solubility in blood, compared with other gases, ensures that its concentration in the alveolar gas will depend solely on alveolar ventilation.

Preliminary studies with xenon 127 were carried out at the Argonne Cancer Research Hospital. The development of the production technology of xenon 127 at Brookhaven is already being funded by the AEC-DAT.

(See Continuation Sheet)

Nuclear Medicine Technology and
Other Health Applications
Early Detection and Localization of
Project Title: Pulmonary Impairment RX-01-03-(f)

16. Expected Results in FY 1973:

This is a new program to be initiated during FY 1975. However, some preliminary studies at BNL have shown that stable inert helium appears to be an excellent tracer for the measurement of closing volumes, and its concentration in the exhaled air can be readily measured with the commercial helium "leak detector". The procedure for measuring distal airway closure is based on a single-breath analysis of the expirate. A bolus of helium is introduced into the mouth of the subject, while seated erect, just after forced expiration to his residual volume (RV). The subject inhales the tracer and air to his vital capacity (VC). A bolus volume of 1 ml is administered to adult males, while a smaller volume is used for female and young male subjects.

All volumes are determined with a spirometer, and the helium concentration in air is measured continuously by withdrawing a small sidestream through a needle valve at the mouth of the subject during forced expiration to the limit of the subject's RV again. The helium concentration in the sidestream is determined with a mass spectrometer of the "leak detector" type. The resultant volumes and helium concentrations are continuously recorded for the entire cycle, and a sharp upward inflection in the helium concentration is observed toward the end of a characteristically straight portion of the concentration-volume curve known as the "alveolar plateau". This inflection point, expressed as percent VC, is believed to indicate distal airway closure.

The introduction of very small boluses -- one of the advantages of using helium -- resulted in a linear increase of closing volume with age for a group of healthy non-smokers. The correlation was in good agreement with that previously reported for argon tracer, but not with those for nitrogen and xenon. Cardiogenic oscillations were greatly reduced with helium in the recorded instrument output, while background noise and statistical fluctuations were damped out by using the leak detector -- in contrast to the output of regular mass spectrometers.

17. Expected Results in FY 1974:

As noted above, this is a new program to be initiated during FY 1975, unless supplementary funds are received to permit an earlier start. In the latter case, the work described below under Section 18 will begin as soon as funding permits.

18. Expected Results in FY 1975:

Airway closure will continue to be measured in large groups of healthy people. The subjects will be drawn mainly from the work force at BNL and patients at the associated hospital centers. Selected patients at the hospitals at BNL and off-site with known lung disorders will also be tested.

(See Continuation Sheet)

18. Expected Results in FY 1975: (Cont'd)

The data, obtained in the form of ratios of closure volume to vital capacity for each subject, will be stored on computer tape and used to correlate airway closure with age, sex, and other vital statistics such as height, weight, and history, including the relationship of the subject to different forms of atmospheric pollution and occupational exposures. Subsequent screening of large groups of people, will compare their closing volumes with the reference value for healthy people. Premature closure is indicative of small airway abnormalities.

A simple and relatively inexpensive package of equipment will be built with assistance from the BNL Instrumentation Division, to provide for the automatic introduction of the tracer and the evaluation of the results. The equipment will: (1) measure the volume of gas inspired and expired from the lungs with a spirometer, (2) measure the concentration of helium in the expirate with a mass spectrometer, and (3) provide for semi-automatic data acquisition and storage on industry-compatible incremental magnetic tape. Analysis of the data would have to be performed on a remote computer. This system of data acquisition would allow an operator to store the single-breath helium washout curve as 1000 sampled points with 8-bits (about 1/4%) resolution. The stored data can be immediately displayed on a Display Unit, and, if satisfactory, the data and appropriate heading identification can be stored on incremental magnetic tape for future analysis. If the curve is not satisfactory, the operator can discard the data and generate a new set of data, recording only the satisfactory curve on magnetic tape.

Computer programs will be written and improvements in analyzing and storing the patient data made. The goal is to store all information on tapes, analyze it by computer operation, and store it for future reference and retrieval.

Other gases, such as hydrogen, nitrogen, neon, argon, sulfur hexafluoride, and xenon, will be introduced -- as stable or radioactive tracers -- individually or in combination with helium, to compare their relative sensitivity and effectiveness with that of helium, and to determine if they offer an advantage over the use of helium alone. The first such gas to be studied will be xenon 127, proposed for use at BNL in related pulmonary ventilation-perfusion studies.

Curie quantities of xenon 127 will be prepared in the BLIP, a unique accelerator facility, for daily use and made available for widespread clinical evaluation at BNL and at the collaborating outside hospitals.

Inasmuch as this program is regarded as a continuing one that does not commence until FY 1975, it may be appropriate to cite here the direction that the work is expected to take beyond FY 1975. The screening of healthy people to obtain reference data on normal lung closure volumes and the

(See Continuation Sheet)

18. Expected Results in FY 1975: (Cont'd)

evaluation of regional lung function with xenon 127 for people with lung impairment will continue.

Since the basic screening procedure will simply indicate the presence or absence of disease, it is hoped to make it more specific by differentiating among the various obstructive lung diseases using trained subjects with respiratory disease. It is thought that the difference in diffusion rates of each gas in a mixed gas tracer, coupled with the effects on diffusion of variations in volumes and tissue elasticity and of gas flow rates resulting from obstructions in the air passages, can be monitored by lung scanning and analysis of the expirate at the mouth to predict the type and extent of obstructive disease. This study will be preceded by work with healthy subjects using artifacts that mimic flow restrictions and other conditions to establish reference conditions. A bench-scale plastic mock-up of pertinent parts of the bronchial tree will be constructed and used to study these conditions outside the body and to predict the in vivo results.

Instrumentation will be developed for precise automatic control and timing of the steps in the process, including helium injection, and on-line data processing, computation, and analysis with a portable mini-computer leading to decisions concerning the patient while he is still present.

19. Description and Explanation of Major Materials, Equipment, and Subcontract Items:

Capital Equipment for FY 1974: None.

Capital Equipment for FY 1975: A low resistance spirometer ($2,100) is required to measure the volumes of air during respiration, a nitrogen analyzer ($2,300) to measure residual volume and the functional residual capacity of the lungs, a residual gas analyzer ($3,400) to measure the concentrations in air of other inert gas tracers besides helium, and a pneumotachometer ($2,100) to measure the flow of air during respiration. Instrumentation ($15,000) will be constructed for the semi-automatic acquisition and storage of breathing data on magnetic tape. (In FY 1976, instrumentation estimated to cost $35,000 will be constructed for on-line data processing, computation, and analysis.)

20. Proposed Obligations for Related Construction Projects:

None.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory — RX-Biomedical & Environmental Research
Laboratory — Program

1. Contractor: Associated Universities, Inc. | Contract No.: AT(30-1)-16 | Task No.:

2. Project Title: Health Effects of Chemical Toxicants and Effluents Determination of Trace Metals in Human Tissues and Their Influence on Certain Diseases | 189 No.: RX-95

3. Budget Activity No.: RX-01-04

4. Date Prepared: May 1973

5. Method of Reporting:
Scientific Meetings
BNL Monthly Letter to AEC
Scientific Journals

6. Working Location: Brookhaven National Laboratory

PRIVACY ACT MATERIAL REMOVED

7. Person in Charge:
G. C. Cotzias
L. K. Dahl

Principal Investigator:
G. C. Cotzias
L. K. Dahl
P. S. Papavasiliou M. Hillman (BNL-DAS)

8. Project Term:
From: To:
To be initiated in FY 1975

9. Man-Years:	FY 1973	FY 1974	FY 1975
Sci., Res. Assoc. (Ph.D. or Equiv.)			3.0
Visiting Sci.			---
Prof. (B.S. or Equiv.)			---
Sci. & Prof. - Total	---	---	3.0
Technical	---	---	3.0
Adm. & Clerical	---	---	0.5
Guests & Research Collaborators	---	---	---
Total	---	---	6.5

10. Costs (In Thousands of Dollars):	FY 1973	FY 1974	FY 1975
Labor (including benefits)			101
Mats., Trav., Dev. Subcont., Spec'l Proc.			11
Reactor, Accel., and/or Computer Usage			0
Allocated Technical Services			3
Gen. & Adm. Overhead			
Total Research Cost	0	0	
Research Division			164
Hospital Division			0
Research Costs	0	0	164
Equipment Obligations	0	0	31

11. Reactor Concept:

12. Materials:

Project Title: Health Effects of Chemical Toxicants and Effluents
Determination of Trace Metals in Human Tissues and Their Influence on Certain Diseases RX-01-04

13. Publications:

Project not yet initiated.

14. Scope:

A) 200 Word Summary:

The generation of power by fossil fuels results in the release of not only vast amounts of carcinogenic hydrocarbons and oxides of nitrogen and sulfur but also trace metals dependent upon the origin of the coal or oil. The effluent industrial gases and liquid wastes introduce large amounts of a wide spectrum of metals, many of which may be toxic and some of which have been shown to be toxic in relatively small concentrations (arsenic, beryllium, titanium, fluorine, cadmium, barium, mercury, lead, vanadium, and bismuth). These elements are alien to the body and can cause various kinds of chronic degenerative conditions. Neither the degree of exposure nor the manner in which the human body must become exposed for disease to develop are understood. The concentration of these materials in human tissues and food has not yet been adequately investigated. This study proposes to determine the concentration of these materials in human tissue obtained from individuals at all stages of life and in addition to begin studies of amnionic fluid, cord blood, fingernail and hair from live newborns and all tissues from stillborns. With the development of highly inbred strains of rats with innate dispositions to develop hypertension or to be resistant to hypertension, and hypercholesterolemia and ultimately atherosclerosis it is possible to critically evaluate the role that is played by cadmium or vanadium in the development of hypertension and atherosclerosis.

B) Supplement to 200 Word Summary:

Elements which are either normal constituents of tissue or essential to life may cause disease either by being scarce or in excess. Such elements are: chromium, manganese, iron, nickel, copper, zinc, and selenium. The concentration of these elements will be studied in tissue samples originating from human beings: displaying symptoms which are similar to those induced in animals by virtue of either excess or deficiency of the elements mentioned; of residents of areas in which industrial and agricultural conditions are such that one can easily suspect gross excesses or marked deficiency in a representative human population. The nutritional and toxicological work involving these metals in experimental animals is sufficiently extensive to permit proper selection of human populations for sampling.

[redacted] technology that exists at Brookhaven for the study of metals is extensive. Chemical techniques and neutron activation analysis would be helpful in specific instances but inadequate for population surveys. X-ray fluorescence is capable of determining, simultaneously, all elements of atomic numbers greater than 15 present in sub-nanogram amounts of tissue. RARAF is ideally suited for the excitation of the characteristic x-ray fluorescence and with on-line computer analysis assays are quick and economical.

(See Continuation Sheet)

14. Scope: (Cont'd.)

In America cardiovascular diseases, cancer, neurological, pulmonary and mental diseases are becoming increasingly important as health problems. Specifically, hypertension involves all ages. At least 10% of adults have hypertension with the incidence progressively increasing in the 5th, 6th, and 7th decades of life up to 20-30%. Among American blacks the incidence is higher. The greatest importance of hypertension ultimately may prove to be its influence on atherosclerosis, the major scourge of Western adult males, and in recent years to an increasing degree in females. In America, the importance of hypertension has been overshadowed by the much higher death rate due to coronary artery disease from atherosclerosis. There is increasing recognition, however, of the fact that hypertension is one of the major risk factors in determining the frequency of clinical coronary artery disease. It is now apparent that approximately half of the patients with clinical coronary artery disease have had antecedent hypertension. The importance of understanding this interrelation, therefore, cannot be overestimated.

It was reported some years ago that food containing a crude sea salt was more effective in inducing experimental hypertension in rats than the same food with an identical concentration of pure sodium chloride. Some experimental and epidemiological data have implicated cadmium as one of the trace metals that might be involved. There are, however, reports suggesting that industrial workers exposed to cadmium do not have a higher prevalence of hypertension than would be expected, but tissues have not been analyzed for cadmium.

The interaction of hypertension and atherosclerosis is vicious. It is known that vanadium inhibits cholesterol biosynthesis experimentally and epidemiologic data suggested that workmen exposed industrially to vanadium had significantly lower mean serum cholesterol levels than that of controls. Manganese, in contrast to vanadium, stimulates cholesterol biosynthesis. It is of further interest that vanadium counteracts the stimulation of cholesterol biosynthesis induced by manganese and that manganese nullifies the depressant action of vanadium on cholesterol biosynthesis. In another research program, 06-03-01-b, hypertensive-prone and hypertensive-sensitive rats have been bred. Substrains of the resistant and sensitive strains of rats have been developed that are genetically predisposed to develop a low or high serum cholesterol on the same high fat cholesterol diet. The availability of these four substrains allows the study of interactions of hypertensinogenic and atherogenic influences in a far more conclusive fashion than heretofore possible. These studies cannot be performed elsewhere since the strains of rats exist only at Brookhaven.

15. Relationship to Other Projects:

The Suffolk County Medical Examiner is interested in these studies and will cooperate in obtaining samples of tissue. In addition, Dr. Marvin Kuschner, Professor of Pathology, State University of New York at Stony Brook, Stony Brook, New York is very much interested in atmospheric contamination

(See Continuation Sheet)

15. Relationship to Other Projects: (Cont'd.)

and the influence of metals upon pulmonary function. He also will contribute substantially in the obtaining of human specimens. The x-ray fluorescence studies of Dr. M. Hillman, BNL (DAS) is essential, related work.

There are many investigators working on trace metals, particularly cadmium. In no instance, however, does anyone else have the unique strain of rats that is used at Brookhaven to study the pathogenesis of experimental hypertension, hypercholesterolemia, and atherosclerosis. These rats provide an unusually sensitive tool with which to study additive factors in diseases of multifactorial causation like hypertension and atherosclerosis.

16. Technical Progress in FY 1973:

Project to be initiated in FY 1975.

17. Expected Results in FY 1974:

Project to be initiated in FY 1975.

18. Expected Results in FY 1975:

It is anticipated that firm design for construction of automated exposure of samples in RARAF, its fabrication and operation would be completed. Neutron activation analysis and chemical techniques would be used for initial study of human tissues. Methods for obtaining fetal biopsy and autopsy material would be established.

The baseline levels of vanadium and cadmium in the four strains of rats mentioned above will be determined. These values will be correlated with cholesterol and triglyceride determinations as well as blood pressures. The effects on these parameters of small quantities of vanadium and cadmium added to the diet will be determined.

At the same time, initial studies will begin on measurements of these two metals in patients with hypertension. A correlation with cholesterol and triglyceride levels will be made.

19. [redacted]cription and Explanation of Major Materials, Equipment and Subcontracts:

FY 1975 Capital Equipment:

Six each flush type rat cage racks would be procured and installed in an animal laboratory for the initial studies ($13,000). An autoanalyzer is necessary to perform the cholesterol and triglyceride determinations that could not be processed on existing equipment ($18,000).

(See Continuation Sheet)

Project Title: Health Effects of Chemical Toxicants and Effluents Determination of Trace Metals in Human Tissues and Their Influence on Certain Diseases RX-01-04

20. Proposed Obligations for Related Construction Projects:

None

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory — Laboratory

RX-Biomedical & Environmental Research — Program

1. Contractor: Associated Universities, Inc.
Contract No.: AT(30-1)-16
Task No.:

2. Project Title:
Effects of Radiation on Living Organisms
Early and Late Effects of Radiation of Different Quality and at Different Dose Rate
189 No.: RX-164

3. Budget Activity No.: RX-03-01-(a)

4. Date Prepared: May 1973

5. Method of Reporting:
Scientific Meetings
BNL Monthly Letter to AEC
Scientific Journals

6. Working Location:
Brookhaven National Laboratory

7. Person in Charge:
C. J. Shellabarger
V. P. Bond
H. H. Rossi

Principal Investigator:
C. J. Shellabarger
L. J. Goodman
A. L. Carsten

8. Project Term:
Continuing
From: To:

9. Man-Years:

Direct Man-Years	FY 1973	FY 1974	FY 1975
Scientific & Professional	1.5	1.0	3.0
Other	10.0	10.5	13.0
Guests & Res. Collaborators	11.5	10.5	11.0
Total	23.0	22.0	27.0

10. Costs (In Thousands of Dollars):

	FY 1973	FY 1974	FY 1975
Research Division	388	367	461
Hospital Division	0	0	0
Research Costs	388	367	461
Equipment Obligations	25	49	36

11. Reactor Concept:

12. Materials:

Project Title: Effects of Radiation on Living Organisms
Early and Late Effects of Radiation of Different Quality and at Different Dose Rate

RX-03-01-(a)

13. Publications:

Tisljar-Lentulis, G. M., Bond, V. P., Robertson, J. S., and Moore, W. H., Jr. The let of 150-MeV protons and 70-MeV negative π mesons near the end of the particle tracks in polyethylene. BNL 50306, April 1971.

50306

14. Scope:

A) 200 Word Summary:

The primary objective of these studies is the accumulation of experimental information for the development and evaluation of theories of radiobiologic action through systematic collection of quantitative data on the effects of radiation on eukaryotic cells and tissues. The effects under study include cell killing, genetic effects, somatic mutation, growth reduction and tumor induction.

The variation in degree of biological effect as a function of the microscopic distribution of energy deposition represents a principal avenue for the understanding of the biological action of radiation; the influence of radiation quality must be accounted for in any theory of the mechanisms involved. Neutrons provide a convenient and powerful tool for varying radiation quality, particularly for the exposure of mammalian systems. A modified Van de Graaff generator makes possible the production of essentially monoenergetic neutrons over a wide spectrum of energies.

Evidence for an association between radiation exposure and breast cancer in the human female continues to accumulate. Data on the neoplastic response of mammary tissue to ionzing radiation in the rat is extensive. The additional research proposed in these studies on the mammary neoplastic response of the rat to ionizing radiation should provide further insight towards an understanding of the qualitative aspects of mammary radiation carcinogenesis in man.

B) Supplement to 200 Word Summary:

The modified Van de Graaff generator at BNL, used originally as the injector for the Cosmotron, is the main feature of the Medical Department's Radiological Research Accelerator Facility (RARAF). The charged particle beams at the facility are capable of direct employment to investigate radiation damage mechanisms at the cellular and sub-cellular level. Precise dosimetric and quantitative biological studies, coupled with microdosimetry and associated methods of theoretical analysis, provide a powerful approach to uncovering the basic mechanisms of interaction of radiation with biological systems. However, most of these studies are also pursued with a view for their practical implications to radiation protection and radiation therapy. Careful control of the various radiation environments produced and the high quality of dosimetric techniques utilized provide a facility uniquely suited

(See Continuation Sheet)

14. Scope: (Cont'd.)

to the intercomparison and standardization of neutron dosimetry methods and instrumentation for radiotherapy and radiobiology as well as for radiation protection. (Bond, Rossi)

The mechanism of action of radiation carcinogenesis in the rat is studied by observing the mammary neoplastic response to chemical carcinogens and radiation in relation to a comparative study of neutron and x radiation; the effect of dose rate; and the interaction of hormonal stimulation of mammary tissue and chemical and physical carcinogens. The dose-response relationships of rat mammary carcinogenesis and exposure to neutrons are studied at neutron dose ranges of 0.125 to 8 rads. The possible "sparing effect" of lowering the dose rate on mammary carcinogenesis is investigated. Various strains of rats are studied for their differing responses to x radiation and chemical carcinogens in regard to the hormonal status of each strain. (Shellabarger)

Another study, investigating radiation effects on the central nervous system is aimed at obtaining basic information on the response of the mammalian central nervous system to x-ray doses in the therapeutic range and studying the comparative radiosensitivity of the elasmobranch and mammalian central nervous system related to differences in structure and function. This information should have practical value in the planning of patient radiotherapy and in furthering the understanding of basic neurophysiology.

In the mammalian CNS studies monkeys, age 12 weeks to 4 years, have received whole and partial brain x-ray exposures to the hand-face area of the cortex as well as the visual cortex in single and multiple doses ranging from 150 to 67,000 rads. Clinical and EEG evaluation are combined with serial sacrifice at post-irradiation periods from 24 hours to 96 weeks. Normal histological procedures and electron microscopy are combined with special techniques of statistical evaluation of alterations in the dendritic plexus and synaptic area to correlate changes in function as measured by the EEG. Special techniques of frequency analysis of photo-evoked response are used in the EEG analysis. In addition, the loss in visual acuity in trained animals is being measured following x irradiation of both visual cortices. A short-term objective is the accumulation of data on the relationship of radiation dose to the pathogenesis of radiation lesions and loss of function. In particular, the dose threshold for decrements in visual acuity and reduction in photo-evoked response is sought. These studies are in collaboration with Roizin, Columbia University; Farrer, Brooks Air Force Base; and Graham, University of Puget Sound.

The morphology of the elasmobranch as compared to the mammal (the location of astrocytes are grouped in intimate contact with vessels in sharks as opposed to being scattered at greater distances in mammals)

(See Continuation Sheet)

14. Scope: (Cont'd.)

enables estimate of the importance of the astrocyte to brain metabolism and as an indicator of radiation damage. The unusual resistance of the shark brain to other injury (particularly to alterations in blood brain barrier) has led to an interest in studying radiation effects in this species. Sharks receive gamma exposures in the 1,000 to 30,000 R range, limited to specific brain areas and are serially sacrificed for histochemical and electron microscopic evaluation. Alterations in the blood brain barrier are evaluated using standard tracer and dye techniques, as well as the newer peroxidase method. Data relating dose and time post-exposure to changes in morphology following gamma irradiation and the measurement of functional changes in the elasmobranch brain will permit comparison of elasmobranch and mammalian responses to radiation in order to understand the importance of differences in structure to varying radiosensitivity. (Carsten)

15. Relationship to Other Projects:

At BNL Sparrow and Smith, Biology Department, investigate the relative biological effectiveness of neutrons of various energies at various doses and dose-rates using primarily plant material. The RBE of neutrons in a wide range of cellular and animal systems is being studied by Bond, and collaborators using the high capability of the RARAF as the source of neutrons. Popenoe and Slatkin, Medical Department, study biochemical aspects of neoplasms using, in part, radiation-induced rat mammary tumors.

At Argonne National Laboratory, Grahn investigates life shortening and carcinogenesis in irradiated mice over the life span; Finkel studies radiation-induced sarcomas of mice and of man with particular reference to the role of a virus in tumor formation. At Oak Ridge National Laboratory, Walberg studies radiation carcinogenesis in mice. Other related studies include those of Rossi, carried on at Columbia University, in addition to those at BNL; Vogel, University of Tennessee on rat mammary carcinogenesis and neutron irradiation; Telles, Bureau of Radiological Health, USPHS on radiation-ethionine mammary carcinogenesis; Huggins, University of Chicago, and Dao, Roswell Park on chemical carcinogenesis using the rat mammary system; and Hempelmann, University of Rochester on the association of radiation and breast cancer in man. (Shellabarger)

[redacted]dies related to research on the effects of radiation on the cent[redacted] nervous system include those of Bruner, Lovelace Foundation, on the d[redacted]lopment of techniques for defining brain function following irradiation via surface and depth recording and stimulation techniques.

Barnes' group at the USAF School of Aerospace Medicine is investigating radiation effects on the equilibrium function in primates, and is particularly interested in the role of damage to the visual system. (Carsten)

(See Continuation Sheet)

Project Title: Effects of Radiation on Living Organisms
Early and Late Effects of Radiation of Different Quality and at Different Dose Rate RX-03-01-(a)

16. Technical Progress in FY 1973:

The Radiological Research Accelerator Facility (RARAF) has been fully developed to provide six target positions for various radiobiology and radiological physics experiments, including a low scatter irradiation environment and a special charged particle physics laboratory. Only minor additions to the instrumentation of some of the new beam lines are required. The transfer of all essential accelerator controls to the new console has been completed. Reliable semi-automatic operation of the Van de Graaff generator has been achieved to simplify routine operation of the accelerator after initial adjustments have been made by an experienced operator. Irradiations are terminated by an ionization monitor after the required dose has been delivered. Neutron-producing targets continue to be replaced periodically as they become exhausted or contaminated. A wide range of neutron energies from a low energy spectrum less than 100 keV to monoenergetic neutrons in excess of 15 MeV have been used to irradiate various systems including Drosophila, Tradescantia inflorescences, Vicia faba seedlings, Osmunda regalis spores, Chinese hamster lung cells, rats and mice. In all of these studies the relation between various effects and the absorbed dose was determined together with the dependence of RBE (relative to 200 kVp x rays) on dose. In the case of Vicia OER as well as RBE was determined as a function of neutron energy. Radiological physics support including design of irradiation techniques and construction of the required fixtures, dosimetry and spectrometry, was provided for all of these studies. Instrumentation has been acquired and is being developed to extend neutron spectrometry down to 2 keV.

A major radiological physics effort was the establishment of a special low-scatter environment essential for a new project involving an international intercomparison of neutron dosimetry sponsored by the ICRU. Special equipment developed for these measurements included precision radiation monitoring instruments, accurate optical alignment fixtures, and a pneumatically-handled 2.5 mg californium-252 source.

Microdosimetry measurements using wall-less tissue equivalent proportional counters to determine event size distributions were completed for simulated tissue sphere diameters of 1, 2, and 4 μm and for 13 neutron energies ranging from a spectrum less than 100 keV to monoenergetic neutrons up to 15 MeV. A comparison of walled and wall-less proportional counter data was also completed using 0.5, 1 and 2 μm sphere diameters and neutron energies of 0.2, 0.5, 2.2, and 15 MeV. The accelerator is also [redacted] used by the BNL Applied Science Department for analysis of trace elem[redacted] in the environment by charged particle proton x-ray fluorescence.
(Bond, Rossi)

Three reports show that fission neutrons have a high RBE for inducing mammary neoplasia in the rat with the suggestion that the RBE for neutrons is higher at low doses than at high doses. To quantitate the RBE for neutrons at various doses, neutron doses of either 1, 4, or 16 rad, produced at RARAF, and x-ray doses of either 80 or 240 R were used. Because of a technical error, the expected neutron energy ranged from approximately

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

0.30-0.43-MeV rather than the desired 0.43-MeV. The experiment was terminated at 60 days after exposure. Even at this short interval, 16 rads produced an incidence of 13%; 4 rads, 7% and 1 rad, 2%, while 240 R of x-ray produced 13% and 80 R was without detectable effect. These results suggested a rather large RBE, hence, another experiment was begun using 0.43-MeV neutrons in the doses of 0.125, 0.5, 2 or 8 rad and x-ray doses of 30, 60 or 90 R. At some eight months after beginning the experiment, the cumulative percent of rats with mammary tumors after 30 R is matched almost exactly by the 0.5 rad dose of neutrons and 0.125 rad has produced more mammary neoplasms than no exposure. Again, a rather high RBE is suggested by these experiments that are still in progress.

To study the possible sparing effect of lowering the dose-rate on rat mammary carcinogenesis, Co-60 gamma radiation was given at dose-rate of 0.03 R per minute at doses of either 88, 265, 530, or 795 R and at a dose-rate of 10 R per minute at doses of either 88 or 265 R. When the experiment was terminated, 340 days after beginning the exposures, the incidence of rats with mammary neoplasia or mammary fibroadenomas was not different at the two dose rates at total doses of either 88 or 265 R. The incidence of rats with mammary adenocarcinomas was not different at 88 R, although there was a somewhat smaller response at 265 R with the lower dose-rate. The dose sparing effect of a lower dose-rate was modest at best under the conditions of this experiment.

It is well known that the Long-Evans strain of rat exhibits a much smaller mammary neoplastic response to either x radiation or dimethylbenz-(a)anthracene (DMBA) than does the Sprague-Dawley strain of rat. To see if the low response of the Long-Evans rat was due to failure of DMBA to reach the target tissue, DMBA was applied directly to mammary tissue in vitro. Preliminary results indicate that Sprague-Dawley rats still show a larger mammary neoplastic response than do Long-Evans rats even after mammary tissue from both strains received directly the same amount of DMBA. These results are interpreted to mean that the strain difference in response to carcinogens does not obtain at the level of mammary tissue-carcinogen interaction.

Rats thymectomized on the day of birth were studied in regard to their mammary neoplastic response to DMBA, but the results were inconclusive due to the poor rate of complete thymectomy. In studying the rate of cell proliferation and/or synthetic activity in regard to mammary neoplasia, prolactin or drugs that increase prolactin secretion were used. Rats x rayed while lactating have shown essentially the same neoplastic response as non-lactating sister rats. Mammary adenofibromas are absent in Lewis rats while Sprague-Dawley rats, similarly treated with x rays or DMBA, have reached a 50+% incidence of rats with mammary adenofibromas. (Shellabarger)

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

In the CNS studies, testing continued on the visual acuity of monkeys that previously received bilateral x-ray exposures in the 500-2,000 rad range. At sixteen months post-exposure no decrement in visual acuity has been observed. Due to budget limitation additional animals have not been irradiated; however, data on the present animals will continue to be collected until they exhibit a decrement or until it is necessary to terminate the study.

The last of the sharks exposed to 30,000 rads were sacrificed and the brains examined for both gross and microscopic changes. The earlier findings indicating essentially no effect from these massive doses of radiation were confirmed.

The feasibility of making electrophysiological measurements on the shark brains using implanted electrodes was investigated. Some base-line recordings were obtained which were encouraging. However, the techniques must be improved before they can be used to determine whether any functional changes take place in the irradiated shark brain. (Carsten)

The genetic effects of neutron irradiation were studied in Drosophila, using the frequency of translocations, sex-linked recessive lethal mutations, and dominant lethal mutations as endpoints. For translocations, the x-ray curve is curvilinear while the neutron curve is linear. The RBE is less than one at all dose levels studied. For sex-linked recessive mutations, the response curve appeared to be linear for both neutrons and x rays, and the RBE of neutrons is less than unity. Using sex-linked recessive lethal mutations, the RBE was determined for cells in various stages of spermatogenesis in order to see if the low RBE values might be due to the shape and small size of the sperm nucleus. The RBE for early spermatids, although less than unity, was no greater than that for mature sperm. Thus the low RBE is not due entirely to the small size of the sperm nucleus nor to the highly condensed state of the chromosomes. Also, extensive work was carried out to verify the work of Per Oftedal indicating that genetic effects of very low doses may be greater than that predicted with extrapolation from high doses.

Unfortunately, this entire program had to be terminated with the untimely death of Dr. Gonzalez. Dr. Abramson has collected and is studying the data obtained by Dr. Gonzalez, and it is hoped that the material can be completely analyzed and reported. It is hoped, also, that means can be found to continue these important studies.

Extensive work, particularly important to testing theories of radiobiological action, have been carried out in the Biology Department using Tradescantia stamen hair test systems (A. H. Sparrow and A. J. Underbrink).

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

These results are reported in the 189 documents from the Biology Department.

The theory group has continued to extend and refine the theory of dual radiation action (A. M. Kellerer and H. H. Rossi). These results are reported in the annual reports of the Radiological Research Laboratory, Columbia University.

17. Expected Results in FY 1974:

The completion of all planned beam lines at RARAF will permit research to proceed more efficiently so that a larger number and greater variety of research projects can be undertaken. The international intercomparison of neutron dosimetry will consist of measurements made at RARAF by a dozen teams of scientists at various intervals during the year. Pending approval by the AEC of a recent proposal by the BNL Health Physics Division, supplementary equipment and staff will be acquired to establish at RARAF a neutron calibration facility to provide outside and local users with standard, well-defined neutron fluences suitable for calibration and testing of radiation instruments and personnel monitoring devices.

Radiological physics studies are in progress in preparation for two new radiobiology experiments. The first will utilize a molecular hydrogen beam to study the biological significance of energy depositions at the nanometer level. The second involves the use of a microbeam to irradiate single cells with one or a known number of charged particles.

Radiobiology experiments will be continued involving neutron energy dependence dose rate dependence and OER for irradiations of Tradescantia inflorescences and Chinese hamster lung cells. The studies on the induction of mammary neoplasms in rats will also be continued. The Drosophila and Vicia faba experiments have been terminated and the data are being analyzed. (Bond, Rossi)

Data will be forthcoming from the Sprague-Dawley rats exposed to neutron doses ranging from 0.125 through 8 rads so that preliminary conclusions can be reached in regard to RBE values for neutron rat mammary carcinogenesis. The August-Copenhagen hybrid rat has been reported to show a synergistic interaction of neutron radiation and exogenous estr[illegible] administration. Lewis rats given either x radiation or DMBA will[illegible] nearing the end of their life span so that any differences between Spra[illegible]Dawley rats and Lewis rats can be documented in preparation for studies on possible hormonal differences in the two strains. Certain antioxidants, widely used in food and commerce, have been claimed to reduce the rat mammary neoplastic response to chemical carcinogens; some antioxidants will be studied in regard to their influence on chemical carcinogenesis and

(See Continuation Sheet)

17. Expected Results in FY 1974: (Cont'd.)

these studies extended to include x radiation. (Shellabarger)

If collaborative support is continued, the effects of multiple exposures on the visual acuity of monkeys will continue to be studied. It is hoped that techniques of electrophysiological measurements in the elasmobranch brain as a parameter for radiation effects will be perfected. If this is not accomplished, an established technique for specific measurements on the olfactory tract will be used after radiation of the olfactory nerves.

Lerner Marine Laboratory may install an x-ray machine. It would then be possible to investigate the effects of radiation on a number of marine organisms which are now impossible with the present Co-60 source designed for narrow beam irradiation of specific areas. (Carsten)

The high RBE's obtained for mammary gland tumor induction have obvious theoretical importance as well as practical implications. Radiation protection guides for radiation workers and for nuclear medicine procedures in which high LET radiations are used perhaps should be reconsidered in this light. In addition, interpretation of the data from Hiroshima and Nagasaki, in which a part of the exposure was due to neutrons, depends very much on the neutron RBE for tumorogenesis as a function of dose. For these reasons, systems in addition to mammary gland neoplasia will be studied. In particular, efforts will be made to begin experiments on myelocytic leukemia induction in mice. This probably will involve a collaborative program between the Medical Department at Brookhaven and the Medical Center at the State University of New York at Stony Brook (Dr. Arthur Upton).

18. Expected Results in FY 1975:

The sophisticated instrumentation and experimental techniques required for the microbeam experiments will require extensive development work. The LET of the traversing particle will be varied by adjusting particle energy. This approach should allow direct observation of the probability of single cell inactivation by particles of different LET, should remove a large area of uncertainty in determining the kinetics of cell inactivation, and should give considerable insight into mechanisms of cell inactivation. It is expected that much progress will be made in perfecting the rather demanding techniques required to perform this type of exposure of sizable population of cells.

Discussions are being held regarding two types of collaborative studies. These include RBE studies of leukemia induction and research in radiation genetics. It appears probable that both of these efforts will be well under

(See Continuation Sheet)

Project Title: Effects of Radiation on Living Organisms
Early and Late Effects of Radiation of Different Quality and at Different Dose Rate RX-03-01-(a)

18. Expected Results in FY 1975: (Cont'd.)

way in FY 1975.

Radiobiological studies designed to test theories of radiobiological action critically will be continued. Studies on the carcinogenic and leukemogenic potential of neutrons particularly at low doses will be continued and extended. (Bond, Rossi)

Carcinogenesis studies with neutrons will be extended to a range of neutron energies in an attempt to relate the RBE for rat mammary carcinogenesis to LET. Additional doses and dose rates of gamma radiation will be studied in regard to the possible sparing effect of lower and lower gamma dose rates. Additional methods of hormonal assay, particularly prolactin, will be developed for use in studies correlating mammary carcinogenesis with hormonal environments. (Shellabarger)

Since all of the radiation-CNS studies are long-term endeavors it is likely that budgetary problems of the Air Force and BNL will severely restrict these studies. (Carsten)

19. Description and Explanation of Major Materials, Equipment and Subcontract Items:

None

20. Proposed Obligations for Related Construction Projects:

None

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory Laboratory	RX-Biomedical & Environmental Research Program

1. Contractor: Associated Universities, Inc.
   Contract No.: AT(30-1)-16
   Task No.:

2. Project Title: Effects of Radiation on Living Organisms
   Radiosensitivity of Immune Responses and Mechanisms of Immune Reactions
   189 No.: RX-174

3. Budget Activity No.: RX-03-01-(b)

4. Date Prepared: May 1973

5. Method of Reporting:
   Scientific Meetings
   BNL Monthly Letter to AEC
   Scientific Journals

6. Working Location: Brookhaven National Laboratory

7. Person in Charge:
   R. D. Stoner
   M. T. Pavlova

   Principal Investigator:
   R. D. Stoner
   M. T. Pavlova

8. Project Term: Continuing
   From: To:

9. Man-Years:

Direct Man-Years	FY 1973	FY 1974	FY 1975
Scientific & Professional	1.0	2.0	4.0
Other	5.0	6.0	6.5
Guests & Res. Collaborators	1.5	1.5	2.0
Total	7.5	9.5	12.5

10. Costs (In Thousands of Dollars):

	FY 1973	FY 1974	FY 1975
[redacted] Research Division	146	197	280
[redacted] Hospital Division	0	0	0
Research Costs	146	197	280
Equipment Obligations	11	15	59

11. Reactor Concept:

12. Materials:

Project Title: Effects of Radiation on Living Organisms
Radiosensitivity of Immune Responses and Mechanisms of Immune Reactions RX-03-01-(b)

13. Publications:

Terres, G., Morrison, S. L., Habicht, G. S., and Stoner, R. D. Appearance of an early "primed state" in mice following the concomitant injections of antigen and specific antiserum. J. Immunol. 108, No. 6, 473-81 (1972). 16711

Hess, M. W., Cottier, H., Sordat, B., Joel, D. D., and Chanana, A. D. The intestinal barrier to bacterial invasion. Presented at the Conference on "A Re-Examination of "Non-Specific" Factors Influencing Host Resistance", Bern-Gurten, June, 1972. 17004

14. Scope:

A) 200 Word Summary:

A goal of this research is to determine the origin, function and fate of lymphoid cellular elements in immune responses. Of primary interest are the highly immunogenic properties of complexes of antigen-antibody as compared with the same antigen administered alone.

Five inter-dependent objectives are explored: (1) radiosensitivity of antibody responses, (2) comparative antigenic properties of complexed antigen and antibody (specific immunoglobulins) in their capacity to elicit early and enhanced primary antibody responses, (3) cellular proliferation in germinal centers of lymphoid tissues during antibody responses, (4) radiation-induced susceptibility to anaphylactic shock, and (5) the genetic control of antibody responses.

Another goal is to determine whether or not tumor virus infections and tumorigenesis are affected by exposure to radiation, and to compare the effects of radiation on in vitro and in vivo virus replication. It is proposed to determine if exposure to radiation will: (1) enhance or depress both in vitro and in vivo infection of cells with DNA and RNA tumor viruses, (2) influence oncogenesis in genetically susceptible and resistant infected chickens, (3) initiate tumors in non-infected chickens, and (4) influence the immune response in genetically susceptible and resistant chickens infected with DNA and RNA tumor viruses.

B) Supplement to 200 Word Summary:

The protection normally conferred to individuals by active immunization with antigens and vaccines and/or passive immunization with specific immunoglobulins in the control of infectious diseases may be abolished after whole-body exposure to sub-lethal doses of ionizing radiation. Exposure of animals to ionizing radiation at various times before, during, and after immunization affords a unique opportunity to study cellular and humoral immune mechanisms as well as the repressive effects of radiation on normal immune responses. Emphasis is directed in these studies to the highly immunogenic properties of complexes of antigen and antibody in eliciting earlier and enhanced antibody

(See Continuation Sheet)

14. Scope: (Cont'd.)

responses, as compared with the same antigen (tetanus toxoid) administered either in soluble or adsorbed form. In order to provide a broad basis for the observed efficacy of primary immunization with complexed antigens, three additional and unrelated antigens, bovine serum albumin (BSA), horseradish peroxidase (HRP) and glucose oxidase (GOX) from Aspergillus niger are used in comparative experiments.

Present findings show a close correlation between an increased number of germinal centers in lymph nodes, as well as their earlier appearance and rapid proliferation of cellular elements, and the appearance of serum antibody. Germinal centers show both an increase in number and proliferate faster when mice are immunized with complexes of antigen and antibody as compared with the same amount of antigen only. Immune defects in so-called immunologic deficiency syndromes in man may result from developmental disorders of immunologically active tissues. Developmental failure of lymphoid tissues may result in a functional deficiency, absence of one or more immunoglobulins, and in morphologically detectable defects. Although germinal centers contain immunoglobulins as shown by immunofluorescent methods, it is apparent that most immunoglobulins lie on the surface of cells or in reticulum cells within germinal centers. Present findings with HRP antigen provide evidence for antibody formation by lymphoid germinal center cells as well as persistence of antigen and/or HRP-antibody complexes between dendritic reticular cells. Increasing emphasis will be given to primary immunization with antigen-antibody complexes prepared with gammaimmunoglobulins (IgG). Complexes of tetanus toxoid and specific human IgG have been prepared for primary immunization studies in man. The long-range purpose of this research is to extend the principles of enhanced immunization demonstrated with the present complexed antigens to situations where antigens of bacterial, viral, protozoan, and parasitic origin are poorly antigenic.

Previous work in this laboratory has demonstrated an increased susceptibility of irradiated animals to fatal anaphylactic shock. Since enhanced antibody responses are obtained in both normal and irradiated mice immunized with antigen-antibody complexes, the anaphylactogenic properties of complexes will be explored when complexes are prepared in antigen excess, equivalence, and antibody excess.

The scope of this research has been enlarged to include a study of the genetic control of antibody responses in many strains of mice. In collaboration with the Jackson Laboratory, Bar Harbor, Maine, more than 140 strains of mice with known genetic constitutions are being made available at no expense. The relative capacity of these various strains of mice to produce primary and secondary tetanus antitoxin responses is being tested. When significant differences in antibody responses are observed, The Jackson Laboratory will develop new recombinant inbred lines for immunologic studies. Immunogenetics has a direct bearing on the genetic control of the gene locus involved in the

(See Continuation Sheet)

14. Scope: (Cont'd.)

observed susceptibility of mice to murine lymphoid leukemia and to rejection of allografts. (Stoner)

Although extensive research interest has been given to the carcinogenic effects of ionizing radiation, little is known about the effects of radiation on latent RNA and DNA tumor virus infections and the influence of radiation on virus-induced tumors. Also, little is known of radiation effects upon the immune system of genetically susceptible and resistant hosts in determining the pathogenesis of tumor virus infections and virus-induced tumors. In this respect, the chicken, with its known indigenous tumor viruses and established genetically susceptible and resistant lines, offers a unique animal model system to study the effects of graded doses of radiation on the virus-host relationship in leukosis. The well-known repressant action of ionizing radiation on immune systems provides an opportunity to assess the relative role of cellular and humoral immunity in resistance to avian leukosis.

The overall significance of the proposed research is that it is possible with this model system to work with known, well-described leukemic DNA and RNA viruses in both susceptible and resistant lines of chickens and to explore the virus-host relationship of leukemia as well as the effects of radiation on the virus-host relationship of leukemia that cannot be studied in man. The vast majority of avian tumors have their histogenesis in the hematopoietic system and have been classified as the avian leukosis complex. A recent classification of the avian leukosis complex has been established by Sevoian as follows:

Avian Leukosis Complex:

LYMPHOID
- Type I (Myxovirus)
  - Subtype A
  - B
  - C
- Type II (Herpesvirus)
- Type III (Myxovirus)

MYELOID
- Type I

ERYTHROID
- Type I

NON-LEUKOSIS TUMORS
- Type I
  - Osteopetrosis
  - Fibrosarcomas
  - Endotheliomas
  - Nephroblastomas, etc.

At least two distinct types of viruses--RNA (RIF-virus, T-virus) and DNA (JM-virus)--can cause leukemic tumors. The etiologic identification of these diseases has led to the terms lymphoid leukosis for RIF-RNA virus tumors, reticuloendotheliosis for T-RNA tumors and Marek's disease for JM-DNA virus tumors. It is estimated that Marek's disease makes up the majority of naturally occurring tumors in poultry. Nearly all field flocks are infected with Marek's disease virus at an early age and remain chronically infected. Though infection levels with Marek's disease are as high as 100% in some cases, neoplasia

(See Continuation Sheet)

14. Scope: (Cont'd.)

resulting from such infections may range from being negligible in some flocks to well over 50% in other flocks. It has also been demonstrated that certain families and lines of chickens are more resistant (or susceptible) to the development of leukemic tumors. That resistance and susceptibility to the avian leukosis in chickens are inherited traits has been demonstrated by Hutt and Cole in selecting over 20 years of sires and dams of families which had a low as well as a high incidence of tumors. However, to date the mechanisms for resistance and susceptibility to leukosis are largely unknown.

The proposed studies will utilize genetically inbred leukosis-susceptible and resistant specific pathogen free (SPF) chickens from the University of Massachusetts, Cornell University, and the Federal Regional Poultry Laboratory, East Lansing, Michigan. Two different types of RNA viruses (Type I - RIF and Type III - T) and one DNA virus (Type II - JM) will be used for inoculation. Gamma and x radiation will be delivered in doses from 10 to 500 rads by whole-body exposure. Avian and mammalian cell cultures will also be irradiated. The antigen-antibody studies will be analyzed by complement fixation, fluorescent-antibody (direct and indirect), immunodiffusion, serum and virus neutralization, and indirect hemoagglutination tests. (Pavlova)

15. Relationship to Other Projects:

Related studies at Brookhaven National Laboratory are those of Cronkite, Chanana, and Joel on Lymphopoiesis and homograft rejection, and of Shellabarger on Radiation-Induced Carcinogenesis. At Argonne National Laboratory, Jaroslow's studies on radiosensitivity of antibody responses, and the studies of Finkel, Reilly, Greco, Rockus, and Dale on Strontium-90 and angiotensin in mice are related. Studies elsewhere include those of: Loan, Storm, Sloan, and McCune at the University of Missouri, Sevoian at the University of Massachusetts; Jansen, Atomic Energy Board, Pretoria, South Africa; Vos, TNO Radiological Defense Lab., The Netherlands; van Bekkum, Balner, de Vries, van Rood, Radiological Institute, The Netherlands; Micklem, University of Edinburgh; Betz and Simar, University of Liege, Belgium; Simic, Slivic, Cirkovic, and Petrovic, Boris Kidric Institute of Nuclear Sciences, Beograd, Yougoslavia; Silverman, University of North Carolina; Fitch and Wissle University of Chicago, Meier, Heiniger, Taylor, Chen, Cherry, The Jackson Laboratory; Martin, Mourer, and Benacerraf, Harvard Medical School; and Herzenberg, Stanford University.

16. [redacted]hnical Progress in FY 1973:

Preliminary results with Cottier et al. (University of Bern) concerning cellular kinetics of lymphoid tissues indicate that more time periods during immunization need to be studied. Secondary tetanus antitoxin responses were elicited in normal and irradiated mice (500 rads) and H-3-histidine was injected on day five after booster injections of fluid toxoid and complexes

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

of toxoid and specific mouse immunoglobulins (IgG). Test mice were sacrificed for tissues and serum 5, 10, 15, 20, 30, 40, 60 minutes and 2, 3, and 6 hours later. Serum antibody levels were determined by neutralization of tetanus toxin. The free-amino acid (H-3-histidine) level was counted in the sera and the tritium-labeled antitoxin precipitated in slight antigen excess with concentrated tetanus toxoid. The antigen-antibody precipitates were washed three times in cold saline and solubilized with Bio-Solv formula BBS-3 (Beckman) for liquid scintillation counting. The rate of incorporation of H-3-histidine into antibody is being evaluated together with an autoradiographic and histologic study of tne lymphoid tissues.

A series of six experiments with 1,260 mice were carried out concerning the anaphylactogenic properties of complexed antigen. The various complexes were prepared in vitro with tetanus toxoid with either isologous mouse antitoxin, specific mouse IgG, or human tetanus immunoglobulin (H-IgG). Actively immunized mice, normal and irradiated, were challenged 6-8 days post-radiation by i.v. and s.c. injections of the complexes and/or tetanus toxoid only. Challenge with antigen only was far more effective in eliciting severe and fatal anaphylaxis than with complexed antigen. When complexes were formed in slight and large antibody excess and injected, fatal anaphylactic shock was not observed. Normally, symptoms of anaphylaxis appear in 5-7 minutes in irradiated mice. When complexes were injected in large antibody excess, the usual symptoms of anaphylactic shock were delayed about 30 minutes, an observation for which there is no explanation.

Studies continued in attempts to account for the highly immunogenic properties of complexed antigens as compared with the same dose of antigens injected alone. The enzymes horseradish peroxidase (HRP) and calf mucosa alkaline phosphatase (APH) were used as test antigens. Both HRP and APH in complex with respective specific antibody elicited enhanced primary antibody responses in mice. Several experiments were carried out using HRP and fluid tetanus toxoid (FTT) in complex with specific immunoglobulin subunits. Porter's methods were used to prepare fragments of specific rabbit IgG to form complexes with the various Fab antibody fragments hoping to learn if the whole or only parts of the molecule will elicit an enhanced antibody response when combined with specific antigen.

A new study was started concerning the comparative hematological and immunosuppressive side effects of chloramphenicol (CAP) and a closely related chemical, thiamphenicol (TAP) in mice. Although TAP is not an approved drug in the United States, more than 15 million people in Europe have been treated with TAP without the appearance of any known cases of aplastic anemia (APA). On the other hand, the rather high incidence of CAP-induced APA has been well-documented. In preliminary experiments, both CAP and TAP

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

inhibited primary tetanus antitoxin responses; after 30 days these animals gave good primary responses. Secondary antitoxin responses were largely unaltered by treatment with either CAP or TAP before the injection of booster doses of tetanus toxoid. During the past year resistance to CAP has been reported for Salmonella typhosa. The analog of CAP--TAP--has similar antimicrobial characteristics. Thus it seems desirable to explore the side effects of this chemical in more depth. Although TAP induces an immediate toxic effect to the bone marrow, it appears to be temporary and normal hematologic findings are seen again within 4-5 weeks.

The study of the genetic control of antibody responses in genetically defined strains of JAX mice has continued throughout the year. The relative capacity of more than 60 strains and inbred lines of mice to produce primary and secondary tetanus antitoxin responses was evaluated. Preliminary results indicate that the various strains may be grouped into three categories: (1) early and high antibody responders, (2) intermediate, and (3) late and low responders. The HRS/J (hairless) strain produced very little antibody after primary immunization. The hairless mouse has a homozygous genotype (hr/hr); the normal mouse, with hair, may have the following genotypes (hr/+) or (+/+). The incidence of leukemia in (hr/hr) mice is 70-80 percent at 18 months, whereas the incidence of leukemia in (hr/+) mice at the same age is only 5-10 percent. It seems apparent that the mutant gene (hr) enhances susceptibility to murine leukemia virus and the wild-type allele (+) induces resistance to leukemogenesis. (Stoner)

The studies on the effect of radiation in tumor virus infection and tumorigenesis are being planned, and equipment assembled. Experiments will be initiated during the latter part of this fiscal year. (Pavlova)

17. Expected Results in FY 1974:

Antibody responses in more than 80 strains of mice from The Jackson Laboratory will be determined. The various inbred strains and recombinant inbred lines will be selected on the basis of previous findings concerning the genetic control of antibody responses. The HRS/J hairless mouse strain will be used in a series of immunological experiments with BSA and HRP as test antigens.

Histological and histochemical studies need to be completed on the localization of HRP, HRP antibody and complexed HRP in lymphoid tissues. The antisera will be tested here and the microscopic evaluation will be completed by Dr. Sordat, Cancer Institute, Lausanne, Switzerland. Enhanced antibody formation following the injection of antigen-antibody complexes formed in antigen excess will be studied in an inbred A/J mouse strain in collaboration with Dr. Habicht, State University of New York at Stony Brook.

(See Continuation Sheet)

Project Title: Effects of Radiation on Living Organisms
Radiosensitivity of Immune Responses and Mechanisms of Immune Reactions RX-03-01-(b)

17. Expected Results in FY 1974: (Cont'd.)

Previous experiments on enhanced responses have been done with either xenogeneic or allogeneic antisera complexed to BSA or FTT. It seems likely that there may be at least two components which account for the enhanced response; one which is effected through the foreign antigenic determinants present on the gamma globulin and one which is effected by the antigen-antibody complex per se. To eliminate the possibility that the enhancement is due to foreign antigenic determinants, studies on enhancement will be performed with isogeneic antisera complexed to the antigen thus studying influence of the complex without possible xenogeneic or allogeneic influences.

A series of experiments will test the hematological and immunological side effects of treating mice with TAP and CAP. These experiments will deal with the immediate toxic effect, comparative dose effects and duration of daily treatment in relation to immonusuppressive effects. Several studies with Cottier et al. on the incorporation of tritiated-histidine into lymphoid cells and specific antibody will continue. (Stoner)

The initial phase of the project on tumor virus infection and tumorgenesis will deal with in vitro studies on the effects of radiation on avian and mammalian cell cultures infected with three tumor viruses: RIF virus (RNA - Type I), T virus (RNA - Type III), and JM virus(DNA - Type II). Fertile eggs from genetically susceptible and resistant chickens to leukosis will be utilized for the preparation of chicken embryo fibroblasts. (Pavlova)

18. Expected Results in FY 1975:

Acquisition of information on the genetic control of antibody responses will allow selection of inbred strains and new recombinant inbred strains for use in study of lymphoid cellular kinetics during antibody responses. The anaphylactogenic properties of antigen-antibody complexes prepared from specific immunoglobulins and enzymatic digest fragments of antibody will be tested in irradiated mice and guinea pigs. Studies will continue concerning the efficacy of immunization of laboratory animals and man with complexes prepared from specific human immunoglobulins. Increased emphasis will be given to poor antigens and particulate antigens. The study of hematologic and immunosuppressive side effects of thiamphenicol and chloramphenicol will be completed. (Stoner)

[redacted] in vitro studies will be continued and augmented by in vivo experiments to determine the effects of various doses of radiation on chickens genetically susceptible and resistant to leukosis. The following tumor viruses will be used: RIF virus (RNA - Type I), T virus (RNA - Type III) and JM virus (DNA - Type II). (Pavlova)

(See Continuation Sheet)

Project Title: Effects of Radiation on Living Organisms
Radiosensitivity of Immune Responses and Mechanisms of Immune Reactions RX-03-01-(b)

19. Description and Explanation of Major Materials, Equipment and Subcontract Items:

FY 1975 Capital Equipment:

Capital equipment requirements include $24,000 for one third interest in the high capacity preparative ultracentrifuge described more fully in RX-03-02-(a). Also, because of shared usage of the autoradiographic grain counter and cyto-analyzing apparatus described in RX-01-03-(d), $25,000 is prorated to this activity.

20. Proposed Obligations for Related Construction Projects:

None

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory
Laboratory

RX-Biomedical & Environmental Research
Program

1. Contractor:
Associated Universities, Inc.

Contract No.:
AT(30-1)-16

Task No.:

2. Project Title:
Molecular and Cellular Radiobiology
The Chemistry and Function of DNA Polymerases

189 No.:
RX-216

3. Budget Activity No.:
RX-03-02-(a)

4. Date Prepared:
May 1973

5. Method of Reporting:
Scientific Meetings
BNL Monthly Letter to AEC
Scientific Journals

6. Working Location:
Brookhaven National Laboratory

7. Person in Charge:
E. A. Popenoe

Principal Investigator:
E. A. Popenoe
D. N. Slatkin

8. Project Term:
Continuing

From: To:

9. Man-Years:

Direct Man-Years	FY 1973	FY 1974	FY 1975
Scientific & Professional	2.0	2.0	2.0
Other	1.5	1.5	1.5
Guests & Res. Collaborators	0.5	---	---
Total	4.0	3.5	3.5

10. Costs (In Thousands of Dollars):

	FY 1973	FY 1974	FY 1975
[redacted]esearch Division	98	113	119
[redacted]ospital Division	6	8	10
Research Costs	104	121	129
Equipment Obligations	4	2	24

11. Reactor Concept:

12. Materials:

13. Publications:

None

14. Scope:

A) 200 Word Summary:

Enzymes which copy the RNA strand of synthetic RNA·DNA hybrid polymers occur in many animal tissues, although the copying of the RNA strand of a naturally occurring hybrid has not been observed. A primary goal is to determine what role RNA directed DNA synthesis plays in the life cycle of the animal cell. The attempt is to isolate in pure form and to characterize an enzyme with this activity from chick embryo tissues, in which it is relatively abundant; also to determine where, within the cell, the enzyme occurs naturally and how the amount of the enzyme may vary during the reproductive cycle of the cell. Synchronized HeLa cells in culture are used in the latter study. A search for a naturally-derived hybrid RNA·DNA polymer which will serve as template for the enzyme is also proposed.

Effort is also directed at the question of whether or not the induction of tumors by radiation is effected by unmasking of a virus. Radiation or dibenzanthracene-induced mammary tumors in rats, and the milk of lactating rats bearing such tumors, will be examined for the presence of "reverse transcriptase" and/or a virus-like particle. The rats might prove a useful model for similar tumors in humans.

B) Supplement to 200 Word Summary:

Some enzymes which synthesize DNA (DNA polymerases) can use RNA templates. In 1970 Temin and Mizutani and Baltimore showed that oncogenic RNA viruses contain an enzyme which will form a DNA chain complementary to template viral RNA. When Spiegelman and co-workers reported that the synthetic polynucleotide hybrid polyriboadenylic acid-polydeoxyribothymidylic acid (rA·dT) is a much more sensitive template-primer for the viral enzymes than is viral RNA, it seemed that a simple test was available for the detection of oncogenic RNA viruses in tissues. However, certain tissue DNA polymerases can also use this template-primer very effectively under the proper conditions. Thus the ability to incorporate radioactive thymidine phosphate into acid-insoluble polymers in the presence of rA·dT template is no proof of the existence of a virus in the tissue. Methods have been developed during the past year for distinguishing whether an enzyme is of viral or tissue origin based on its preferences among several synthetic polynucleotide templates.

The advantage of RNA-directed DNA synthesis to an RNA tumor virus is obvious, but what purpose does it serve in normal cells? The suggestion has been made that information transfer from RNA to DNA by enzymes of this type may play a role in cell differentiation or in gene amplification. Evidence suggesting that this might be true has been obtained in the case of the amplification of the genes for ribosomal RNA which takes place during the

(See Continuation Sheet)

14. Scope: (Cont'd.)

maturation of frog oocytes.

As stated above an effort is underway to isolate and characterize at least one enzyme with RNA-directed DNA synthesis from chick embryos. When the RNA directed-DNA polymerase has been isolated and purified it may be possible to develop or discover specific enzymatic inhibitors which would be of great help in determining its function in vivo.

An attempt will be made to discover possible naturally-derived hybrid polymers which would serve as template-primers for the polymerase. (At present the only known hybrids with which the enzyme will function are synthetic polynucleotides.) Among possible naturally derived hybrids which might function in this capacity are hybrids of chick embryo messenger or ribosomal RNA with chick embryo DNA, the product of the action of RNA polymerase on chick embryo DNA, and chromatin isolated from chick embryo nuclei which is reported to contain RNA.

A second research effort is directed at the general question of whether the induction of tumors by radiation (or by chemical carcinogens) is effected by the unmasking of a virus which may be present within the cell. Particles which appear to be identical to the type B mouse mammary tumor virus have been observed in human milk and in human mammary carcinomas. These particles have been detected with high frequency in the milk of women with a family history of breast cancer, but with low frequency in the milk of women without such family history. Type C particles have been isolated from a transplantable rat mammary adenocarcinoma derived originally from a spontaneous tumor which appeared in an aged Sprague-Dawley rat. Although these particles are capable of bringing about a transformation of rat mammary gland cells in tissue culture, there is no convincing evidence that they are able to produce mammary tumors when inoculated into young rats. Further, it has not proved possible to induce tumors by permitting young rats to nurse from mothers bearing radiation-induced mammary tumors.

Radiation- or dibenzanthracene-induced tumor tissue and the milk of lactating rats bearing such tumors are studied for "reverse transcriptase" associated with a 70S RNA and/or with a particle of density about 1.16 g/cc, characteristic of RNA viruses. An electron microscopic search for such particles will be undertaken. If evidence for the occurrence of virus-like particles is obtained, efforts will be directed to the question of whether their presence, or the appearance of "reverse transcriptase" is useful in predicting which rats in an irradiated group will develop tumors. If successful the rat model may become very useful for determining factors that may be revelant to human breast cancer.

Some studies have already been carried out on a hybrid template-directed DNA polymerase from rat mammary tumors (see below). This polymerase is

(See Continuation Sheet)

14. Scope: (Cont'd.)

presumed to be of tissue rather than viral origin. These studies should enable one to distinguish clearly between "reverse transcriptase" of viral origin and similar tissue-derived enzyme activity.

A third research effort is contemplated in collaboration with Dr. Wolf Prensky, Sloan-Kettering Institute. A method of labeling RNA with Iodine-125 developed at Brookhaven National Laboratory Medical Department by Dr. S. L. Commerford, produces RNA with much higher specific activity than attainable by any other method. Dr. Prensky has used this method to study the specific hybridization of (labeled) ribosomal 5S RNA with metaphase chromosomes of drosophila and some other species, locating the genes for 5S RNA by radioautography.

The RNA of oncogenic RNA viruses will be labeled by I-125. Then a radioautographic search will be made for sequences within cellular DNA which are complementary to viral RNA. Cells transformed by the virus as well as non-transformed cells will be studied, and cells transformed by one virus will be studied for complementarity to RNA from other viruses. Although most investigators have found some complementarity between viral RNA and cellular DNA, one cannot be sure from a review of the literature whether the complete viral genome is present in transformed cells. Technically these experiments will be difficult but the collaboration with Dr. Prensky using his automated equipment will significantly increase the sensitivity of the procedure so that one or two grains per cell can be measured. Calculations show that introduction of only one I atom per 100 bases in RNA will give more than 100×10^6 dpm/μg, which is the highest specific activity attainable using all four tritium labeled RNA precursors, but nowhere near the maximum possible with I-125.

15. Relationship to Other Projects:

Other laboratories studying RNA directed DNA synthesis in chick embryo tissue include those of Chargaff at Columbia University, and Chapeville at Faculte des Sciences, Paris.

Reports from these two laboratories are in conflict and differ from BNL data. Weissbach, at Roche Institute of Molecular Biology has reported on a similar enzyme (or enzymes?) from HeLa cells. This group is not studying the variations in enzyme level with the cell cycle. Their data in part have been shown to be in error at BNL.

Mammary tumors and viruses in mice, are extensively studied. Whether these studies are relevant to genesis of human breast cancer is uncertain but is studied intensively by Moore, Institute for Medical Research, Camden, New Jersey; and Spiegelman, Columbia University, Chopra, National Cancer Institute, and Shidlovsky, Pfizer, Inc., Maywood, New Jersey, have studied

(See Continuation Sheet)

15. Relationship to Other Projects: (Cont'd.)

the virus-like particle originally derived from a spontaneous rat mammary tumor. At BNL a unique opportunity exists to study, on a molecular level, radiation induced tumors produced in another study (Shellabarger RX-03-01-a).

16. Technical Progress in FY 1973:

From chick embryo skeletal muscle (legs), the hybrid template-directed DNA polymerase has been purified approximately 100 fold by a three-step procedure involving ammonium sulfate fractionation, DEAE cellulose chromatography, and phosphocellulose chromatography. During the course of this purification other DNA polymerases which prefer as template "activated" or denatured DNA are removed.

Although it was believed a year ago that two different enzymes--one soluble, the other membrane-bound--occur in the chick embryo, this opinion is no longer tenable. During the course of the above purification the enzyme in the soluble fraction and the enzyme subsequently extracted by Triton X-100 behave more and more the same. There is now no reason to believe that the two preparations differ in any important way.

When first extracted from the tissue the enzyme appears to have a very high molecular weight (perhaps 2×10^6, determined by gel filtration). With purification the apparent molecular weight becomes smaller, passing through one or more intermediates, so that after the phosphocellulose step the MW is about 30,000. The nature of this transition and the exact size of the purified enzyme is still under study.

Methods for purification of nuclei of various chick embryo tissues have been worked out and preliminary studies on the subcellular distribution of hybrid-dependent DNA polymerase activity have been made. Considerable variation has been observed from tissue to tissue. For example, in liver, 58% of the total activity was found in the cytoplasm whereas in heart tissue more than 90% was in the cytoplasm. The significance and generality of this observation is under continuing study.

As another part of the effort to understand the biological significance of hybrid template-dependent DNA polymerases, fluctuations in enzyme level have been studied in synchronized HeLa cells. Again, enzyme activity was found both in the nucleus and cytoplasm. Variation in total cellular enzyme level was small compared to variation in the rate of DNA synthesis. During a period of rapid DNA synthesis (S phase), enzyme specific activity (cpm incorporated/mg protein) was approximately 50% higher than before G_1 or after G_2 DNA synthesis. During S phase, while the rate of DNA synthesis doubled, there was no significant change in enzyme specific activity. However, a larger proportion of the total enzyme activity was found in the nuclear fraction during S phase than in G_1 or G_2.

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

Chromatography on DEAE-cellulose reveals at least two hybrid template-dependent polymerases in HeLa cell extracts separable from DNA dependent polymerases and a terminal transferase.

Last year attempts to detect hybrid template-directed DNA polymerase in mammary tumor tissue from dibenzanthracene-treated rats were unsuccessful. Now in a limited number of cases some activity has been found in these tissues. Preliminary results indicate that activity in the rat tumor tissue is confined primarily to the cell nucleus, in contrast to chick embryo heart where about 90% of the activity appears in a "cytoplasm" fraction. The significance of this observation is not apparent at present.

The anticipated studies on hybrid-dependent polymerases in lymphocytes, described a year ago, have not been carried out because sufficient personnel was not available and because significant progress in other laboratories made this seem like a less promising area for our efforts than those areas actually pursued.

17. Expected Results in FY 1974:

A real understanding of the function of hybrid-dependent polymerase in chick embryo tissues can probably only be attained with a pure enzyme. For this reason the purification will continue to be pursued using such techniques as: improved ion exchange chromatography using gradient elution, density gradier centrifugation, chromatography on DNA-cellulose, and, possibly, preparative disc electrophoresis. In addition to using specific activity as a measure of purification, the purification will be monitored by analytical disc electrophoresis, electrofocusing and analytical ultracentrifugation. A major problem in purification has been instability of the enzyme as purification proceeds. A concentrated effort will be made to find conditions preventing loss of enzyme activity. Instability prevents an accurate determination of the molecular weight of the purified enzyme.

The search for a natural heteropolymer which would serve as template-prim for the reaction will be pursued more vigorously.

With the HeLa enzyme, standardization by DEAE cellulose chromatography or phosphocellulose chromatography will be undertaken to detect if there are fluctuations in the amount of the two (or more?) hybrid template-dependent polymerases with the cell cycle.

Tissues from radiation- and dibenzanthracene-induced tumors will be examined for their ability to incorporate tritiated thymidine (from tritiated thymidine triphosphate) into particles of approximately 70S. Since the amount of incorporation at best would be expected to be small, this study will be

(See Continuation Sheet)

17. Expected Results in FY 1974: (Cont'd.)

carried out on a sufficient number of samples to be sure that the results, either positive or negative, are reliable. If incorporation into 70S particles is found, the incorporated activity will be examined in the ultracentrifuge by isopycnic banding to see if it is in a particle of density approximately 1.16 g/cc (characteristic of a virus). The incorporated activity will also be examined by established hybridization techniques to determine whether or not it is complementary to any of the known and obtainable oncogenic viruses. Finally, the various samples under study will be examined in the electron microscope for the presence of virus-like particles.

Progress in the study of hybridization of I-125 labeled viral RNA to cellular DNA will depend on how much time Dr. Prensky can devote to it. It is anticipated that at least one complete study can be made. This will probably involve the interaction of Murine Leukemia Virus (Rauscher) with BALB/c mouse bone marrow cells.

18. Expected Results in FY 1975:

Although the study of the hybrid template-dependent DNA synthesis in chick embryo tissues has not progressed as fast as was anticipated a year ago, it is reasonable to expect that purification of the enzyme involved will be completed during the coming year. Since the mechanism of DNA replication in eukaryotic cells is still very obscure, the role of this particular enzyme in the cell's economy still requires investigation. The ability to prepare a specific antibody to the purified enzyme should facilitate this study. In this area of research, the outlook changes so rapidly that it is difficult to predict what the emphasis will be a year or two from now.

The studies proposed on radiation-induced mammary tumors will probably require more than one year. If the presence of type B or C particles in these radiation-induced tumors is established, animals which have been irradiated--or treated with chemical carcinogens--will be examined to determine whether particles are present prior to the appearance of detectable tumors. If a careful search fails to show the presence of virus-like particles in these rat tumors, the studies will terminate.

Studies on virus RNA hybridization will be extended to other viruses and other cell lines to see if some generalizations can be made.

19. [redacted]scription and Explanation of Major Materials, Equipment and Subcontract Items:

FY 1975 Capital Equipment:

A high capacity preparative ultracentrifuge is necessary for large scale preparations of viruses, and will be used in other research activities and intracellular particles

(See Continuation Sheet)

19. Description and Explanation of Major Materials, Equipment and Subcontract Items: (Cont'd.)

FY 1975 Capital Equipment: (Cont'd.)

(RX-03-01-b and RX-03-02-c) of the Medical Department as well as by investigators in the BNL Biology Department. The cost of the temperature controlled basic unit with sample feed system, titanium rotor bowl and accessories is $72,000 which has been equally distributed to three budget activities in the amount of $24,000 to RX-03-02-a, RX-03-02-d, and RX-03-01-b.

20. Proposed Obligations for Related Construction Projects:

None

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory — Laboratory

RX-Biomedical & Environmental Research — Program

1. Contractor: Associated Universities, Inc.
Contract No.: AT(30-1)-16
Task No.:

2. Project Title:
Molecular and Cellular Radiobiology
Effects of Radiation and Chemicals on Control of Hemopoiesis
189 No.: RX-224

3. Budget Activity No.: RX-03-02-(b)

4. Date Prepared: May 1973

5. Method of Reporting:
Scientific Meetings
BNL Monthly Letter to AEC
Scientific Journals

6. Working Location:
Brookhaven National Laboratory

7. Person in Charge:
E. P. Cronkite
V. P. Bond
A. D. Chanana
Principal Investigator:
A. L. Carsten E. P. Cronkite
A. D. Chanana
H. Burlington, Mt. Sinai University, N.Y.

8. Project Term:
Continuing
From: To:

9. Man-Years:

Direct Man-Years	FY 1973	FY 1974	FY 1975
Scientific & Professional	2.0	3.0	2.5
Other	11.0	12.5	13.5
Guests & Res. Collaborators	4.5	6.0	6.0
Total	17.5	21.5	22.0

10. Costs (In Thousands of Dollars):

	FY 1973	FY 1974	FY 1975
Research Division	246	334	355
Hospital Division	43	48	52
Research Costs	289	382	407
Equipment Obligations	54	47	65

11. Reactor Concept:

12. Materials:

Project Title: Molecular and Cellular Radiobiology
Effects of Radiation and Chemicals on Control of Hemopoiesis RX-03-02-(b)

13. Publications:

Sordat, M., Sordat, F., Cottier, H., Hess, M. W., Riedwyl, H., Chanana, A. and Cronkite, E. P. Studies on lymphocytes. XV. Analysis of the in vivo division cycle of large lymphoid cells in calf thoracic duct using combined microspectrophotometry and autoradiography. Exptl. Cell. Res. 70, 145-53 (1972). 16035

Nieto Garcia, M., and Johnson, H. A. Cell proliferation kinetics in goldfish acclimated to various temperatures. Cell Tissue Kinet. 5, 331-9 (1972). 16466

Johnson, H. A., and Pavelec, M. Thermal noise in cells. A cause of spontaneous loss of cell function. Am. J. Pathol. 69, No.1, 119-29 (1972). 16876

Cronkite, E. P. Kinetics of granulopoiesis. Presented at the Gesellschaf Fur Nuclearmedizin E.D., Freiburg, Germany, September, 1972. 17282

Chanana, A. D., Schaedeli, J., Hess, M. W., and Cottier, H. Predominance of theta-positive lymphocytes in gut-associated and peripheral lymphoid tissues of newborn mice. J. Immunol. 110, No. 1, 283-5 (1973). 17033

14. Scope:

A) 200 Word Summary:

The broad objective in this research is an understanding of the injurious effects of radiation, drugs and chemicals on hemopoiesis, and the processes of recovery from these effects. Knowledge from these studies should improve understanding of diseases of hemopoietic cell differentiation such as leukemia, hypoplastic anemia, lymphoma, myelofibrosis, polycythemia vera, and thrombopenic and granulopoietic states. Description of the cell-to-cell interactions within bone marrow, definition of the feedback loops controlling differentiation into cell lines, and a quantitative measurement of the kinetics of cell proliferation is attempted in order to define the chemical basis of regulation of new blood cell production. These mechanisms must be determined in order to define how the genes in the hemopoietic stem cell (HSC) are switched on and off to maintain the size of the stem cell pool, replete it and control differentiation and amplification upon demand for blood cell production. A battery of techniques are adapted or developed for studies of hemopoiesis following irradiation or administration of drugs and chemicals to animals, and in normal and diseased states of animals and human beings.

B) Supplement to 200 Word Summary:

Among the techniques utilized in these studies are: hemopoietic cell growth in diffusion chambers; in vitro bone marrow and blood cell culture; splenic colony growth from the HSC; cell kinetics using tritiated

(See Continuation Sheet)

14. Scope: (Cont'd.)

thymidine and P-32 labeled diisopropylfluorophosphate (DFP-32); depletion by extracorporeal irradiation of the blood (ECIB); Fe-55 suicide of erythropoietic precursors; and production of humoral regulators of hemopoiesis in vivo in renal glomerular tissue culture systems.

Fe-55 suicide, recently developed here, allows the near-total obliteration of that segment of erythropoiesis which is incorporating iron into hemoglobin. The short path length of Fe-55 Auger electrons (about 1 μm) results in deposition of almost all the energy within the cell boundaries of the red cell precursors. Thus with a sufficient amount of Fe-55, these cells are killed--eliminating new production of red cells while the HSC continues to flow down the erythropoietic pathway. If there is an intramedullary feedback loop from the differentiated red cell compartment to the pluripotent HSC which senses diminished red cell production before there is anemia, an increased flow of the HSC into erythropoiesis would be expected with an initial diminution in size of the HSC pool, measureable in mouse by reduction in number of colony forming units in the spleen (CFU_S). The relative proportion of pluripotent HSC's that are dormant or actively in cycle is measured by killing the cells in cycle either by hydroxyurea or by suicide through incorporation of tritiated thymidine. The quantitative effect of these treatments is measured by CFU_S or a limiting dilution analysis of the number of stem cells growing in diffusion chambers.

A combination of these techniques applied serially to mice receiving continuous Fe-55 suicide will be used to determine the rate at which the HSC is diverted down the erythropoietic pathway and whether the HSC has a limited capacity for mitosis as stated in the Hayflick hypothesis or an infinite capacity, a question in chemotherapy of human malignant diseases.

Erythropoiesis is regulated in part by the hormone erythropoietin (EP), and α_1 glycoprotein. To date EP has been obtainable only by extraction from the blood plasma or urine of severely hypoxic or anemic human beings or animals. The development of renal glomerular cultures from goat and human kidneys synthesizing EP, assayable in the supernate, will allow biochemical study of the factors that may control production of EP at the cellular level (pO_2, pCO_2, pH, androgens, etc.) and will permit purification and characterization of human EP. Studies will then be directed to the production of antibodies against EP and development of radioimmune assays for clinical application. If purified radiolabeled EP, produced by adding amino acids labeled with carbon-14 or tritium to the culture, has specific activity high enough for autoradiography, attempts will be made to identify the site of genetic control of erythropoiesis by combining autoradiography and karyotyping.

Granulopoiesis is studied in the intact mammal utilizing labeling with tritiated thymidine, determining the DNA content of the cells in various stages of granulopoiesis, and determining the changes in the

(See Continuation Sheet)

14. Scope: (Cont'd.)

ratio of mitotic figures and DNA labeling at the different levels of maturation from myeloblast through the myelocyte. These studies are performed before and after the induction of severe inflammation in order to derive insight into the mechanism by which the organism increases the production of granulocytes upon demand. In vivo studies in animals and man are complemented by studies involving the growth of human and animal bone marrow and peripheral blood cells in diffusion chambers implanted into the peritoneum of the mouse in a xenogenic system. An autologous system has been developed in the goat whereby the goat's own peripheral blood or marrow cells are grown in multiple diffusion chambers implanted in its own peritoneal cavity. The growth curves for proliferating and non-proliferating granulocytes from normal bone marrow and from peripheral blood cells are measured in terms of the total cell numbers and the rate of progression through the proliferative and differentiated stages of granulopoiesis. Bone marrow and peripheral blood cells from patients with a wide range of hematopoietic disorders are cultured before, during, and after therapy to gain an insight into the disease state.

Bone marrow transplantation may be life saving after fatal irradiation or after aplasia induced by drugs. To date, allogeneic transplantation of marrow though partly successful, carries the risk of severe and sometimes fatal graft vs. host disease because of a lack of histocompatability between donor and recipient. The only safe and really effective transplantation is between identical twins. Since the CFU_S and the HSC are believed to be identical, reported observations here of an increase in CFU_S grown in diffusion chambers, suggests that one might be able to grow useful amounts of HSC's that could be frozen and stored for later use. Development of culture replication of HSC's will be attempted.

The regulation of platelet production and the preparation and preservation of platelets for transfusion remain important for treatment of radiation casualties. Inhibitors and stimulators of platelet production are studied when time and facilities permit. Radiation hemorrhage correctable by transfusion of compatible homologous platelets remains impractical because of inadequate preservation techniques. At the Blood Research Laboratory, United States Naval Hospital, Chelsea, Massachusetts, Dr. Valeri is studying the viability and preservation of platelets using normal volunteers. Platelets are prepared, preserved with various techniques and are transfused into individuals prior to administration of aspirin, and their viability studied by determining whether the increased bleeding time induced by the aspirin is shortened by the autotransfusion of the preserved platelets. The ultimate test of platelet viability and function is the prevention or treatment of thrombopenic bleeding. Collaborative studies at BNL have been planned on in vivo studies of the hemorrhagic state in animals to correlate the output of red cells in the cannulated thoracic duct with thrombopenia and the decreased output of red cells after transfusion of preserved platelets.

(See Continuation Sheet)

Project Title: Molecular and Cellular Radiobiology
Effects of Radiation and Chemicals on Control of Hemopoiesis RX-03-02-(b)

14. Scope: (Cont'd.)

Studies in man are aimed at elucidation of the defect in the leukemic population of cells compared to normal cell proliferation. Leukemia is studied by a combination of techniques: measurement of cell kinetics following administration of tritiated thymidine; serial sampling of the bone marrow and peripheral blood; culture of leukemic cells in diffusion chambers implanted into the mouse complemented by *in vitro* culture; and measurement of DNA content (when facilities are acquired). The enzyme profile and the karyotopes of chronic granulocytic leukemia cells are studied before and after culture of cells in diffusion chambers.

Radiobiological studies are concerned with determination of the D_o of the human HSC required for radiation therapy and space flight and the relative biological effectiveness (RBE) of various neutron energies on the CFU_S.

15. Relationship to Other Projects:

The studies on lymphopoiesis reported in RX-01-03-(d) are a closely related research program.

Elsewhere, related studies include those of Lewis, Trobaugh, Fried and Knospe, St. Luke's Hospital, University of Illinois, on factors in the bone marrow and spleen which support hematopoiesis, and the hemopoietic inductive micro-environment in the spleen and bone marrow. Till and McCulloch, Department of Biophysics, University of Toronto, the initiators of the splenic colony work, are concerned with short- and long-term feedback loops regulating growth, differentiation of the stem cell, the effects of drugs on the stem cell and the relationship of the stem cell to leukemia.

Stohlman and Quesenberry, St. Elizabeth's Hospital, Brighton, Massachusetts apply splenic colony formation, erythropoietin administration, *in vitro* bone marrow culture, and more recently the diffusion chamber technique learned at BNL, to study growth kinetics and factors related to erythropoiesis and granulopoiesis. Benestad, Breivik and Boyum, Oslo, Norway, the developers of the diffusion chamber technique for study of hematopoiesis, continue its application to ascertain the nature of the factors which influence stem cell proliferation and differentiation.

[redacted]inson, University of Colorado Medical Center, uses the *in vitro* grow[redacted]f murine and human blood bone marrow cells in patients with various blood dyscrasias and in studying the regulatory factors concerned with granulocytopoiesis.

Rothstein, Athens, and Cartwright, University of Utah, apply the *in vitro* culture of bone marrow and blood cells along with a modification of the millipore diffusion chambers to study stem cell proliferation, differentiation, and factors regulating granulocytopoiesis.

(See Continuation Sheet)

Project Title: Molecular and Cellular Radiobiology
Effects of Radiation and Chemicals on Control of Hemopoiesis

RX-03-02-(b)

16. Technical Progress in FY 1973:

Fe-55 suicide of differentiated erythropoietic cells was demonstrated and the relationship of Fe-55 dose to suppression of erythropoiesis was established. In addition, it was shown that suppression of erythropoiesis resulted in a diminution of the number of CFU_S's, suggesting that the flow of stem cells into erythropoiesis had been increased, partially depleting the CFU_S's in the marrow. Since depletion results in increased mitotic rate it may be possible to test whether the HSC has limited or unlimited capacity to divide.

Initial studies on the determination of the D_o of human stem cells irradiated in vitro and grown in diffusion chambers were completed. The D_o determined for all granulocytic cells is about 115 rads and for proliferating granulocytic cells approximately 70 rads.

In studies on growth of murine bone marrow cells in diffusion chambers it was shown that a humoral factor diffuses into the chambers stimulating the proliferation of the HSC as indicated by the increase in number of CFU_S's and yield of total granulocytic series. Also, growth was shown to be greater in the irradiated than in the non-irradiated host. These observations of FY 1972 were confirmed in continued experiments, but since there may be xenogeneic effects (human cultures in the mouse) and allogeneic effects (non-inbred cultures in the mouse), an autologous system of diffusion chamber culture of hemopoietic cells was developed using goats. Bone marrow is removed from the goat, prepared and introduced into diffusion chambers. After the goat is irradiated in the Medical Research Reactor gamma chamber the diffusion chambers are implanted into its peritoneal cavity and subsequently harvested at pre-determined intervals. There is excellent growth of the granulocytic series and macrophages, but lesser growth of lymphopoietic and erythropoietic cells. This system eliminates histocompatability problems on cell proliferation. Since large amounts of plasma and peritoneal washings are obtainable in the goat, production and isolation of the factor that diffuses from the irradiated animal into diffusion chambers and stimulates hemopoiesis is being studied.

Upon demonstration that renal glomerular cultures of human and goat kidneys would produce EP, a grant was obtained from NIH to further these studies. All cultures of goat glomeruli produce EP. However, in three cultures made from separate human kidneys, one produces EP and the other two produce colony-stimulating factor (CSF), an agent that increases the yield of granulocytic colonies in in vitro bone marrow culture. Glomerular cultures continue to produce EP for over one year even though the monolayers have been replaced by thick layers of cells. When trypsinized, resuspended, and sub-cultured in almost the total absence of the original glomeruli, these cultures of epithelial cells with rare glomeruli continue to produce EP. Dr. Havran, Mount Sinai University School of Medicine, has commenced column separation and purification of EP from supernates of glomerular cultures.

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

Bone marrow and peripheral blood cells combined with EP-producing cells from glomerular cultures are now cultured to see if the intimate mixture of EP-producing cells with the HSC will initiate active erythropoiesis.

Studies relevant to granulopoiesis confirmed that a humoral factor diffuses from the irradiated host into diffusion chambers stimulating growth of granulocytic cells, CFUs, and macrophages. Intermittent hypoxia during the culture period reduces the yield of granulocytic cells and macrophages which suggests competition for the HSC common for erythrocytes, granulocytes, and macrophages. Study of the growth of the HSC was extended to the culture of normal concentrates of human peripheral blood in the diffusion chamber. Significant growth was observed only in irradiated mice.

Unidentified and largely lymphocytoid blast cells increased exponentially during the first eleven days in culture; during the first 2-3 days the number of small lymphocytes decreased and then remained constant. This raises the question as to whether the decrease in the small lymphocyte population is the result of transformation and proliferation into the large lymphocytoid blast cell. Granulocytes decrease rapidly during the first 5-6 days in culture, approaching zero levels. At 8-9 days, proliferating eosinophils and neutrophils appear, followed within 24-48 hours by an increase in numbers of non-dividing eosinophils and neutrophils. In older cultures there is a striking increase in the number of plasmacytoid cells which appear at a time when the large blast cells are diminishing in number, suggesting a relationship between the two. The time parameters of cell proliferation in the diffusion chambers, after labeling with tritiated thymidine, were shown to be a little faster than those observed in earlier in vivo studies.

The measurement of the DNA content of myelocytes (in collaboration with Killmann and Ernst, University of Copenhagen), showed that approximately 62% of myelocytes have a 2n DNA content, indicating that they are either in the G_1 phase or have gone out of cycle on their way to becoming metamyelocytes. This fulfills the first requirement of the BNL model for granulocytopoiesis.

Studies were initiated on the question of whether myelocyte cycle time is shortened at the expense of the G_1 period by inflammation, thus allowing time for another mitosis with an increased output of neutrophils. Preliminary results in dogs show: 1) in the normal steady state small myelocytes behave like 2n DNA cells (not labeled by tritiated thymidine); 2) the "flash" labeling index is increased by inflammation; 3) myelocytes progress through the cell cycle and replace metamyelocytes more rapidly.

17. Expected Results in FY 1974:

Fe-55 suicide studies will continue with emphasis on study of the apparent intramedullary feedback loop between the differentiated erythropoietic compartment and the HSC responsible for accelerating the flow of

(See Continuation Sheet)

17. Expected Results in FY 1974: (Cont'd.)

cells into erythropoiesis. Hypertransfusion of red cells in the intact mouse suppresses input of HSC into erythropoiesis. If the HSC flows into the erythropoietic pathway in the plethoric mouse after Fe-55 suicide continues, it will prove the existence of the proposed intramedullary feedback loop. In addition, a large number of mice will be treated with Fe-55 to induce continued erythropoietic suicide in order to accelerate cell division at the HSC level and to test the validity of Hayflick's hypothesis in the intact animal.

It is planned to ascertain the D_o of human HSC's in marrow from a sufficient number of individuals to establish the statistical variation.

Attempts will continue to produce the diffusible factor that influences growth in the diffusion chambers by exposing goats to whole-body irradiation followed by plasmaphoresis. Activity will be sought first in the α_1-glycoprotein fraction where CSF and EP are found. Studies will also be performed to see if extracorporeal irradiation of the blood alone will suffice to produce CSF, EP, and the factor that stimulates growth of allogenic, autologous, and xenogenic blood and bone marrow cells in diffusion chambers.

Studies on EP will continue with emphasis on determining the optimal pCO_2, pO_2, pH, and exogenous androgens on production of EP in glomerular cultures. It is hoped to develop suspension cultures or large scale monolayer cultures to produce large amounts of EP for purification, fractionation, and the production of antibodies against EP. If the latter is successful the anti-EP immunoglobulins will be separated; and using fluorescent microscopy it is hoped to identify the EP-producing cell in the culture. If large scale production of EP is successful, radioactive amino acids will be added to the culture in order to produce radioactive EP for autoradiographic studies of its cellular and subcellular localizations. In collaboration with Dr. Charles Saladino, Adelphi University, the ultrastructure of the glomerular cultures will be studied from time of preparation of the glomeruli and at regular intervals after being placed into culture.

A microspectrophotometer must be obtained in order that systematic studies on DNA content, cell proliferation, and tritiated thymidine labeling of the normal and leukemic cells in culture may be continued as this phase of the work can no longer be performed for us at the University of Copenhagen.

Attempts will again be made to produce large numbers of autologous HSC's utilizing the system developed by Dr. Laissue to grow autologous cells in diffusion chambers implanted in an animal's peritoneum. Marrow will be fractionated to eliminate the differentiated cells. The residual undifferentiated cells will be put into diffusion chambers and implanted into the peritoneum of the mid-lethally irradiated goat and grown for the maximum period of time before there is discernible cell differentiation. At this

(See Continuation Sheet)

17. Expected Results in FY 1974: (Cont'd.)

time the chambers will be removed; the animal given fatal whole-body irradiation and its HSC's grown in culture harvested from the diffusion chambers and then injected into the goat to test protective ability.

Human blood leukocytes will be fractionated into "pure" cell types to see whether the HSC present in blood will grow and differentiate in the absence of mature cell types. With this model system one should be able to evaluate the influence of cell-to-cell interaction in addition to the long-range and long-duration feedback loops from the periphery to the stem cell. In analyzing the preceding, DNA content, sequence of tritiated thymidine labeling, and cytology will be observed.

Further studies on granulopoiesis will be performed before and after the induction of inflammation at various time intervals in order to dissect the sequence of events at the myelocyte level and the HSC level that determine the increased production of granulocytes.

Studies on the growth of leukemic cells and cells from other blood dyscrasias will continue as clinical material is available.

18. Expected Results in FY 1975:

Fe-55 suicide will continue--the actual use of this new technique depending upon the results obtained in FY 1974. The studies on D_o of human stem cells will probably be completed. Studies on the diffusion chamber factor and determination of whether it is identical to the CSF and/or the leukocytosis-inducing factor (LIF) will continue if the problems are not resolved in FY 1974. The autologous culture of bone marrow cells in animals will continue in an endeavor to determine the importance of cell-to-cell interaction by implanting diffusion chambers into diverse organs. Attempts will be made to develop long-term monocyte cultures as a source of CSF, LIF, and diffusion chamber stimulatory factor.

EP production in human glomerular cultures should be sufficient for the production of adequate amounts of antisera for radioimmuno-assay in patients. It is hoped to produce radioactive EP with sufficient specific activity suitable for autoradiography. When satisfactory radioactive EP is available it will be utilized in vitro, in erythropoietic cultures, and in vivo to study metaphase of erythropoietic cells. The genetic locus of EP production may be identified in this manner. Consideration will also be given to a pilot plant for production of human EP for possible clinical application.

Studies on the role of inflammation and the mechanism by which it increases cell proliferation may terminate. Studies on cell proliferation in leukemia will continue. The studies on platelet transfusions and control of thrombopoiesis in collaboration with Valeri will probably be reactivated.

(See Continuation Sheet)

Project Title: Molecular and Cellular Radiobiology
Effects of Radiation and Chemicals on Control of Hemopoiesis RX-03-02-(h)

18. Expected Results in FY 1975: (Cont'd.)

It is hoped to develop the diffusion chamber technique as a means of studying the mechanism of action of known hematopoietic depressant drugs.

If personnel and time permit attempts to grow diverse human cancers in diffusion chambers will commence with the objective of assaying the effect of radiation and chemotherapeutic agents on the cancer cell growth.

19. Description and Explanation of Major Materials, Equipment and Subcontract Items:

FY 1975 Capital Equipment:

Miscellaneous equipment requirements for this budget activity are estimated at $20,000 and include an input and retrieval terminal, two cyto-centrifuges, microscope, and a portable laminar flow enclosure. $20,000 is allocated as one half share in the blood cell separator reported in Paragraph 19 of RX-01-03-d; and $25,000 is prorated to this activity for one quarter interest in the autoradiographic grain counter and cyto-analyzer reported in RX-01-03-d.

20. Proposed Obligations for Related Construction Projects:

None

(See Continuation Sheet)

Molecular and Cellular Radiobiology
Effects of Radiation and Chemicals on Control
Project Title: of Hemopoiesis RX-03-02-(b)

Reiteration of Assurance Statement
on
Investigation Involving Human Subjects
Including Clinical Research

The Hospital of the Medical Research Center, Brookhaven National Laboratory, complies with the policy for the protection of human subjects participating in activities supported directly or indirectly by grants or contracts from the Department of Health, Education, and Welfare and from the Atomic Energy Commission. In fulfillment of its assurance, this institution has established a standing committee to review investigations involving human subjects, including clinical research, and said committee is charged with responsibilities to determine for each proposed study that:

the rights and welfare of each patient will be adequately protected,
that the potential benefits outweigh the risks, and
that an informed consent will be obtained from each patient.

The committee, appointed by the Director, Brookhaven National Laboratory, provides for independent judgment of its members, and alternates are provided to serve when there may be conflict of interest. This institution encourages constructive communication between the committee and principal investigators on the safeguarding of the rights and welfare of subjects; will provide the required care for persons injured as a result of participation; and maintains complete documentation of proposals and reviews. Approved investigations are reviewed by the committee at least annually and the institution periodically reviews its practices and procedures to assure they are consistent with the policies published by the Department of Health, Education, and Welfare.

As of March 7, 1973, the Institutional Review Committee for review of Medical Department research activities involving human subjects is composed of:

J. S. Robertson, M.D., Chairman
G. C. Cotzias, M.D., Alt. Chairman
S. H. Cohn
E. A. Popenoe, Alt. for Dr. Cohn
G. Chikkappa, M.D.
H. Connell
R. A. Love, M.D.
G. Price
N. P. Rathvon, Jr.
D. N. Slatkin, M.D., Alternate
A. P. Wolf, Alternate
L. K. Dahl, M.D. - Clinical Consultant
W. A. Finn - Administrative Consultant
C. W. Flood - Health Physics Consultant

Signature: Eugene P Cronkite

Title: Chairman, Medical Department

Date: March 7, 1973

RX-234

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SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory
Laboratory

RX-Biomedical & Environmental Research
Program

1. Contractor:
Associated Universities, Inc.

Contract No.: AT(30-1)-16

Task No.:

2. Project Title:
Molecular and Cellular Radiobiology
Mechanisms of Action of Free Radicals and Hormones

189 No.: RX-235

3. Budget Activity No.:
RX-03-02-(c)

4. Date Prepared:
May 1973

5. Method of Reporting:
Scientific Meetings
BNL Monthly Letter to AEC
Scientific Journals

6. Working Location:
Brookhaven National Laboratory

7. Person in Charge:
D. C. Borg
I. L. Schwartz (Mt. Sinai School of Medicine)

Principal Investigator:
D. C. Borg
I. L. Schwartz (Mt. Sinai School of Medicine)
R. W. Walter (Mt. Sinai School of Medicine)

8. Project Term:
Continuing

From: To:

9. Man-Years:

Direct Man-Years	FY 1973	FY 1974	FY 1975
Scientific & Professional	4.0	4.0	4.0
Other	2.5	3.0	4.0
Guests & Res. Collaborators	4.0	5.0	5.0
Total	10.5	12.0	13.0

10. Costs (In Thousands of Dollars):

	FY 1973	FY 1974	FY 1975
Research Division	149	169	207
Hospital Division	28	32	39
Research Costs	177	201	246
Equipment Obligations	35	15	25

11. Reactor Concept:

12. Materials:

Molecular and Cellular Radiobiology

Project Title: Mechanisms of Action of Free Radicals and Hormones RX-03-02-(c)

13. Publications:

Le Fevre, M. E., Dox, L. J., and Brodsky, W. A. Metabolism of depleted turtle bladder. J. Membrane Biol. 8, 205-18 (1972). 16677

Glass, J. D., Schwartz, I. L., and Walter, R. Bridging of peptides to solid supports through the dinitrophenylene moiety: bidirectional extension of peptide chains. J. Am. Chem. Soc. 94, 6209-11 (1972). 16896

Borg, D. C., Fajer, J., Forman, A., Felton, F., and Dolphin, D. Pi-radical ions from the oxidation and reduction of chlorins, chlorophyll and bacteriochlorophyll. Presented at IVth International Biophysics Congress, Moscow, August 1972. 17254

Glass, J. D., Walter, R., and Schwartz, I. L. Bidirectional peptide synthesis on a solid support. Presented at the 12th European Peptide Symposium, Halle, East Germany, September 1972. 17699

Le Fevre, M. E., Dox, L. J., Mc Clain, O. M., and Brodsky, W. A. Induction of tissue swelling by alteration of orientation of tissue sacs. Comp. Biochem. Physiol. 41A, 319-25 (1972). 16195

Le Fevre, M. E. Effects of ouabain and high K+ on respiration of turtle brain and urinary bladder in vitro. Comp. Biochem. Physiol. (in press) 17309

Le Fevre, M. E., Reincke, U., Arbas, R., and Gennaro, J. F. Lymphoid cells in the turtle bladder. Anatom. Rec. (in press) 17560

14. Scope:

A) 200 Word Summary:

Free radicals are involved in a number of fundamental biological reactions, especially those of oxidoreduction. Radiobiological effects, including sensitization and protection, depend largely upon competitive free radical reactions. Free radicals are implicated in some of the interactions of pollutants and carcinogens with tissue constituents. Many reactions of oxidative metabolism involve free radical pathways, as do the properties of some hormones and drugs. This program investigates free radicals as reactive intermediates in normal and pathological metabolism by seeking to identify and characterize free radicals found in tissue and biological substances. Present projects study radiation and radiation-like chemical reactions of biomolecules and correlate these with reactions of pollutants and carcinogens. Magnetic resonance spectroscopy (EPR) provides foundations for most experimental work on free radicals. The Laboratory has proficiency in studying tissues and short-lived biochemical free radicals by EPR, having pioneered on-line computer applications to this research.

(See Continuation Sheet)

14. Scope: (Cont'd.)

In studies on hormone actions mild and versatile methods of solid phase peptide synthesis are developed. These methods, based on previous studies in peptide hormone chemistry, physiology, and pharmacology, allow for preparation of sophisticated peptide hormone analogs for use in research and in chemotherapy.

B) Supplement to 200 Word Summary:

Free radicals are essential components in many bioenergetic mechanisms. Despite the development of a substantial understanding of free radical reactions in physical-organic and polymer chemistry, the elaboration of general theories of free radical behavior in physiological chemistry and the documentation of special free radical intermediates remain patchy and incomplete. The need to detect small numbers of free radicals in biomedical experimentation has led to the widespread application of electron paramagnetic resonance (EPR) spectrometry, because EPR and related magnetic resonance techniques can identify and describe free radicals with great sensitivity and specificity. Its absolute sensitivity is often insufficient, however to detect the low residual levels of free radicals persisting in surviving tissues.

The BNL program investigating bioenergetic free radical mechanisms has utilized EPR and a special high-velocity flow apparatus for maintaining steady states of labile free radicals in liquid reaction systems. Computerization, new radical-trapping procedures, ion-irradiation techniques, and special means of examining large samples of frozen tissue have been explored. Application of ELDOR (ELectron-electron DOuble Resonance) to biomedical and irradiated samples is about to begin, ENDOR (Electron-Nuclear DOuble Resonance) applications are planned. Also, collaboration is planned with the Physics and Chemistry Departments to carry out EPR spectroscopy while samples are being irradiated by an electron beam from the Dynamitron accelerator.

One project is directed toward radiation and radiomimetic chemical reactions on bilogical target molecules: correlations with reactions of pollutants and carcinogens. This involves EPR studies of gamma irradiation and atom bombardment of nucleic acid constituents and identification of some hydrogen-addition and -abstraction radicals, using both amorphous and polycrystalline dry samples and some single crystals of nucleic acid bases and related compounds. Hydroxyl radicals formed by chemical reduction of hydrogen peroxide will be employed in special flow apparatus developed at BNL for high frequency EPR (35 GHz, Q-band). Extension from radiation-like reaction on bases, nucleosides, etc. to oligonucleotides and possible to polynucleotides and nucleic acids is foreseen. Other anticipated extensions include: i) Application of ELDOR and ENDOR to irradiated biochemicals in the solid state; ii) Projection of the EPR/flow work to include interactions of free radicals of some carcinogens and pollutants and of peroxy compounds with nucleic acids and their constituents and with cell membranes and their lipid components; iii) EPR observation of radiation-produced free radicals in liquid and tissue

(See Continuation Sheet)

14. Scope: (Cont'd.)

of radiobiological or biochemical interest during electron beam irradiation, contingent upon development of a BNL interdepartmental EPR/irradiation facility.

Free radical forms of porphyrins appear to be important intermediates in some of the redox reactions of heme enzymes. Closely related chlorin and bacteriochlorin free radicals are involved in the photochemical steps of photosynthesis. One project evaluates the significance of porphyrin radicals in biological electron transport collaboration with J. Fajer (BNL Department of Applied Science), R. H. Felton (Georgia Tech.) and D. Dolphin (Harvard). This work is crucial to the BNL free radical program in establishing a firm theoretical and experimental basis for free radical research.

Another project is directed at the nature of free radical signals intrinsic to tissue or those induced by carcinogens, pollutants, drugs, disease or radiation. Since 1954 undefined and unidentified EPR signals have been obtained from "surviving" (fresh but not actively metabolizing) tissues. These signals are believed to represent paramagnetic complexes containing three components: 1) nitrogen oxide-like moieties from the xenobiotic (i.e., exogenous) molecules or their metabolites, 2) sulfur ligands from endogenous biological sites (quite possibly ferredoxin-like electron acceptors in cellular drug and aryl hydrocarbon mixed-function oxidase systems), and 3) iron atoms from the P-450 or P-448 cytochrome enzymes in the oxidase systems. Thus the EPR spectrum obtained from tissues during the latent period of chemical carcinogenesis may not reflect the neoplastic process but the metabolism of a cellular "defense reaction" against the carcinogenic agent. Non-neoplastic drugs may be inducers of tissue mixed-function oxidase enzymes and may give rise to these EPR signals.

Special apparatus was developed at BNL to identify the paramagnetic sites in surviving tissues which give rise to the "normal" singlet-type EPR signals. Further development of these procedures, plus supplementation with computer processing of data and with ELDOR and ENDOR spectrometry is planned. If identification of tissue paramagnetic sites is attained correlation with exposure to pollutants, different disease states, particular drug therapies, and the effects of ionizing radiations will start.

ELDOR and ENDOR are complementary not competitive double resonance spectroscopies for amplification of EPR in research on free radicals and other paramagnetic centers. Some of the unresolved or incompletely analyzed spectra will be studied by ELDOR and ENDOR. Recent achievement of ENDOR capability by others underscores the need for ENDOR equipment if BNL is to remain competitive.

Free radical reactions associated with enzyme and hormone actions will be aimed at investigation of some enzymic and pyridine nucleotide free radicals

(See Continuation Sheet)

14. Scope: (Cont'd.)

by pulse radiolysis with identification of putative free radicals of DPN and TPN coenzymes and related substituted nictinamide model compounds, and their possible involvement in enzyme reactions. (Borg)

Research collaborators from the Department of Physiology of the Mount Sinai School of Medicine, have been working for some time to correlate the primary structure and conformation of neurohypophyseal hormones with their physiologic functions and metabolic fates. The various studies undertaken have lead to a proposed model for the conformation of oxytocin in solution, to a demonstration that certain distinguishing structural features of the neurohypophyseal hormones may serve as recognition sites for specific hormone-inactivating enzymes, and to a rationalization of many structure-activity functions. In order to prepare sophisticated hormone analogs for research and for therapeutic purposes, reflecting these insights, it has been necessary to devise new methods of peptide synthesis. In particular, new mild methods are being developed for the removal of amino-protecting groups and for the release of completed peptide products from solid supports in the solid phase method of peptide synthesis. (Schwartz,Walter)

15. Relationship to Other Projects:

EPR has been used primarily in chemistry and physics, but applications to the life sciences have been increasing rapidly. It is not feasible to summarize here all of the work in these areas alone, nor are all the projects presently supported known to us. Moreover, the widespread occurrence in nature of free radical intermediates also makes relevant to this program a great volume of published work using procedures other than EPR; and it is not possible to review this material comprehensively either.

However, on the basis of the known projects and the literature it is concluded that the instrumental developments in the BNL program involve little overlap with work elsewhere, and the major subject areas of BNL's biomedical work on free radical mechanisms are not directly duplicated. (Borg)

The researches in solid phase peptide synthesis carried out at this laboratory are an integral part of a concerted effort to determine the molecular mechanism of hormone action and to apply such knowledge to medical purposes. Although these studies are designed and undertaken with specific applications in mind, the techniques being developed are of very broad applicability both in peptide synthesis and in other areas. The approach here in modifying peptide synthetic procedures is considered unique and is not known to be pursued in other laboratories. (Schwartz,Walter)

(See Continuation Sheet)

Molecular and Cellular Radiobiology

Project Title: Mechanisms of Action of Free Radicals and Hormones RX-03-02-(c)

16. Technical Progress in FY 1973:

The new EPR spectrometer (Varian E-12) along with improved ELDOR was installed and interfaced with the on-line computer system and is operative.

The old EPR spectrometer, retained for high-frequency (Q-band) and continuous flow work, was upgraded through the installation of a newer model, reconditioned magnet control. A new and more successful two-stage flow/mixer was designed and fabricated for Q-band EPR experiments on free radicals involved in radiation and chemical carcinogen reactions with DNA constituents and other biochemicals. True separation of the two mixing reactions finally was achieved in a Q-band configuration. (as pointed out in other years, the Q-band apparatus significantly conserves reactants in comparison with "standard" x-band EPR flow, a critical consideration for many of our proposed applications.) Although useful, the new mixer's individual stages were slower than the best one-stage mixers, and further design improvements may be possible.

Computer programs for resolution enhancement of spectra have previously been incorporated in the data system; however, for spectral lines of gaussian shape (which are common in this work) the available filter functions can provide only an approximate match. This has been satisfactory in most cases, but analysis of certain incompletely resolved spectra has been confounded by adventitious lines which are produced when spectral features are strongly sharpened by unmatched shape functions. Proper matching for gaussian lines requires Fourier transformation of either the data or the filter function, and a Fortran program incorporating modified fast Fourier transform algorithms has been written for the CDC-6600 to perform gaussian sharpening. After further modification the program can be incorporated directly into the on-line computer system.

In additional work, crystals of gamma-irradiated deoxyadenosine monohydrate (dADNS) and 1-methyl thymine (1-MT) were aligned by x-ray crystallography, and x-band EPR data in many planes were taken and computer processe Some Q-band EPR studies also were carried out on 1-MT, as anticipated. An estimated 80% of the EPR measurements were carried out, and incomplete analys are in hand. These indicate temperature-sensitive radical chains in the case of 1-MT, but the hoped-for study of 1-MT (and a co-crystal of 1-MT and 9-meth adenine) irradiated and examined at low temperature could not be accomplished because funds for completion of a liquid-nitrogen irradiation apparatus were not available.

Partial analyses of several crystals of irradiated dADNS reveal multiple free radicals, one or more of which are not completely stable with time (weeks and data from all crystals are not yet fully reconciled. Furthermore, the single crystal spectra seem more complex than those previously published on dADNS, so final identification has not been made of those spectral components

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

due to free radicals from the sugar (deoxyribose)moiety. Nevertheless, there is already further support for last year's proposal that resolution enhancement of EPR "powder spectra" might provide identification of sugar radicals in sample configurations of more general radiobiophysical use than the single crystal. Computer-enhanced spectra from irradiated polycrystalline dADNS were equivalent to those obtained from crushing an irradiated single crystal, and pending final confirmation upon complete analysis of the single crystal, the dominant EPR features of a sugar radical are now assignable in the resolution-enhanced powder spectra.

Radiomimetic chemical reactions wherein the hydroxyl radical is produced by chemical reduction of peroxide were studied further, using the Q-band flow systems. Most experiments continued to use Ti(III) as the initiating reductant, but the proposal to evaluate the faster $Cr(II)/H_2O_2$ reaction was commenced, using 6-methyl uracil as the test substrate. Preliminary results indicated some improvement over $Ti(III)/H_2O_2$ reactions, but the system proved combersome and time-consuming, so reaction conditions were not optimized and further explorations were postponed in light of the limited time left available for Schmidt and the uncertainty of his replacement. However, with the Ti(III) system much of the work anticipated last year was carried forward. The first reports of EPR spectra from hydroxyl radical reactions on bases and nucleosides of nucleic acid purines were further refined. Sugar and base radicals are evident from purine nucleosides. Halogenated bases give rise to markedly different spectra. Adenine derivatives deuterated at C8 and run in deuterated solvent revealed only slight line narrowing, indicating little unpaired electron delocalization on carbon atoms with dissociable protons or on C8 (this last finding contrasts with significant C8 interaction in hydrogen-addition radicals of adenine compounds, as previously reported).

Toward the year's end the expected completion and testing of the new two-stage flow/mixer for Q-band EPR allowed a study on transient free radicals from carcinogens and pollutants and their reactions with DNA constituents and other biological target molecules. A detailed EPR spectrum was obtained by dithionite reduction of the carcinogen 4-nitroquinoline-1-oxide (4-NQO) and it strongly resembled that reported by Nagata from ultraviolet irradiation of the more proximate carcinogen form, 4-hydroxyaminoquinoline-1-oxide. Although reaction of the radical with one-electron oxidants was observed, no reaction was detected with reductants or with nucleic acid constituents. Hence this free radical does not react in the fashion expected for the ultimate carcinogen. This is not surprising in view of the present understanding that electrophilic forms of carcinogens (such as certain epoxides or cationic free radicals) form adducts at nucleophilic sites of nucleic acids, because the radical obtained from 4-NQO by dithionite reduction was electron-rich, so any carcinogenic radical that might be obtained from 4-NQO derivatives would have to be a different form.

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

Work by Schmidt on cytological studies on cell cycle control in collaboration with J. Van't Hof (BNL Biology Department), was undertaken to acquire proficiency in techniques that would be useful in the radiation/carcinogenesis project. Initial research involved assessment of colchicine effects on DNA synthesis and cell cycles in root meristems of the bean Vicia faba, using tritiated thymidine autoradiographic methods. Further work during the past year showed the colchicine effects had little dependence upon the stage of the cell cycle. Therefore the emphasis shifted to sunflower (Helianthus annus) meristems synchronized by sucrose starvation. Most cells were halted in G1 with others in G2 and a few "leaking" through. Attempts were made to cause the major population in G1 to move and accumulate in G2 following a pulse of sucrose in order to verify applicability of the Principal Control Point hypothesis to G2 in this species. Initial results were inconclusive.

In collaboration with Fajer and others on free radical forms of porphyrins EPR analysis of tetraphenylbacteriochlorin cation radicals was completed following preparation of selectively deuterated derivatives by Dolphin but only after unanticipated difficulties. These stemmed from failure of certain deuteration reactions to go to completion, leaving a mixture of product free radicals following oxidation, but the complex spectra were analyzed by computer techniques. Only confirmatory experiments are now required to complete this phase of the work.

Plans to map further the delocalization of unpaired electron density in anion radicals of porphyrins and related compounds were advanced significantly. Anion radicals of porphyrins, bacteriochlorins and chlorophylls were prepared coulometrically and chemically, and were examined optically and by EPR. These radicals included anions from chlorophyll, C-13-chlorophyll, bacteriochlorophylls and deuterated bacteriochlorophylls. From a comparison of their properties with those of the chemically unidentified "primary" electron acceptor components of photosynthetic organisms it was possible to rule out chlorophylls for that role in photosynthesis.

Proposals to determine homogeneous rates of electron transfer between porphyrins and their radical ions by NMR line broadening and to measure magnetic susceptibilities by the Evans technique were indefinitely postponed because of BNL's deficient NMR capabilities. The hoped-for ELDOR investigations were precluded by the delayed arrival and installation of the equipment, but are now imminent; and a single pilot ENDOR run during a visit to another laboratory confirmed the applicability of that double resonance spectroscopy to investigations on porphyrin free radicals.

Further elucidation of the pathways of electron transfer through model porphyrins was achieved in collaborative kinetic studies of Forman with

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

N. Sutin (BNL Chemistry Department), using stopped-flow and temperature-jump methods. Work reported last year on the reduction of ferric iron complexes (other than porphyrins) catalyzed by various bridging ligands suggested electron transfer to the central metal ion by way of the ligand's π electrons, a conclusion consistent with numerous indications from our porphyrin research program that redox changes of certain heme enzymes (such as cytochrome c) may proceed by way of free radical forms of their equatorial porphyrin ligands. However, additional progress this past year on acid-soluble tetrapyridyl derivatives of iron porphyrins gave evidence of direct reduction of the central metal ion, so if heme enzymes shuttle redox equivalents through their porphyrins, major effects on the electron pathway must be exerted by solvent and/or by the axial ligands. (Borg)

In studies on mechanisms of hormone action BOC-cysteine was successfully attached to solid supports and recovered by thiolysis, using essentially the methods developed for histidine peptides. Presently model peptides are being synthesized by this method, using special conditions required by the S-dinitrophenyl group situated in the beta position from the carboxyl of cysteine. In addition work began on development of enzymatically removable blocking groups for the amino group. Fundamental and broadly applicable new methods were worked out in the process of preparing the requisite intermediates for this project, and a practical scheme of enzymatic deprotection seems to be evolving. (Schwartz, Walter)

17. Expected Results in FY 1974:

With the acquisition of a second spectrometer and ELDOR, a specially modified signal-averager was ordered to provide accessory signal digitizing capability. It will require some modification of the present computer set-up to allow data transfer from this equipment with format compatibility so that it may be used as a means of data access to the main system. The Fortran computer programs for resolution enhancement of gaussian spectral lines by Fourier transform methods should be completed early next year. Subsequently machine-language versions will be developed for inclusion of the procedure in the on-line computer system.

The ELDOR apparatus, although partially operative on strong test samp[redacted] requires further check-out and minor correction by the manufacturer. In a[redacted]ion, some modifications of the computer system will be required for full [redacted]R compatability. Application to EPR signals from tissues remains promi[redacted]g, although difficult, but it is unlikely to proceed during this next year without additional personnel. Considering the progress of other laboratories, it will not be practical to await procurement of ENDOR apparatus.

(See Continuation Sheet)

17. Expected Results in FY 1974: (Cont'd.)

Therefore preliminary ENDOR investigations will be carried out when a demonstration ENDOR spectrometer is available for a few weeks at Varian's New Jersey applications laboratory. Pending those trials, collaborations with other laboratories with ENDOR capability may be set up.

The new two-stage mixer for continuous flow work with Q-band EPR is relatively slow, difficult to set up, and not regularly consistent in performance. Further improvement is a goal, but with limited time remaining for Schmidt to complete on-going work and delay in obtaining a replacement the work is only tentatively scheduled.

EPR data collection for analysis of the crystals of irradiated deoxyadenosine-H_2O and 1-methyl thymine should be completed, and full interpretation of the computer-enhanced powder spectrum of irradiated dADNS is expected. The $Ti(III)/H_2O_2$ radiomimetic reactions of the hydroxyl radical on purine derivatives, studied with Q-band flow methods, will be pursued.

Oxidative chain reactions involving dissolved oxygen and lipoperoxy free radicals as chain carriers may be involved in the damage reactions of several classes of pollutants, as well as of some drugs, radiation, and other potentially noxious agents. Furthermore, "normal" aging mechanisms and membrane changes associated with malignant transformation of cells may be mediated, in part, through such reactions; and the mixed function oxidase enzymes that both detoxify and activate some pollutants and other carcinogens, as well as many drugs, are membrane-bound systems that produce free radicals capable of damaging both their own and other intracellular membranes. Preparations are being made to extablish methodologies for lipid extraction from membranes of animal cells, for mixed function oxidase (aryl hydrocarbon hydroxylase) assays, for lipoperoxide assay by luminescence and fluorescence spectroscopy (as well as by nonspecific and indirect means such as thiobarbituric acid reaction with malondialdehyde), for product analysis by thin-layer and gas-liquid chromatography, and for synthesis of spin-labeled lipids. Experiments to test some of the effects of mixed function oxidase activity and of carcinogen and pollutant action on nuclear DNA, as mediated through the nuclear membrane will use nuclei isolated from livers of rats pretreated with tritiated thymidine. Following a modified McGrath and Williams centrifugal separation of DNA on an alkaline sucrose gradient, strand breakage will be determined from the tritium distribution as one measure of deleterious effects on the DNA itself.

Another assay of nuclear "damage" will be the incorporation of lipid spin labels into the nuclear envelope monitored by EPR. A modification of this approach designed to evaluate enzymic activity of mixed-function and other

(See Continuation Sheet)

17. Expected Results in FY 1974: (Cont'd.)

oxidases in membranes will determine by EPR the incorporation of spin-label precursors (free radical "traps" such as appropriate nitroso compounds which can be oxidized to relatively stable nitroxide radicals in situ). Plans are also being considered to utilize non-linear EPR techniques and ELDOR to follow slow motions and possible redox reactions of spin labels incorporated in membranes both with and without stimuli for oxidative chain reactions.

In studies on free radical forms of porphyrins the cation radical work and the anion radical experiments on metallotetraphenylbacteriochlorine will be completed but extension of the mapping of electron spin densities of porphyrin cation and anion radicals is planned. Certain tetramethyl- and propyl-substituted porphyrins synthesized by Adler of New England Research Institute will be studied to resolve some remaining ambiguities in analysis and EPR splitting assignments. The use of metal isotopes with nuclear spins (Fe-57, Zn-67, Mg-25, Cd-113, etc.) remains an attractive possibility. The interesting effects of counterions on porphyrin radicals previously reported, and the relationship of these effects to heme enzyme function may be pursued further.

The establishment of a conjoint Brookhaven facility for on-line EPR spectrometry during ionizing irradiation will be attempted. Nearly ten years ago Fessenden set up at Carnegie-Mellon a facility that permits EPR to be done on steady-state populations of transient free radicals produced by an electron beam from a 3-MeV Van de Graaff accelerator. This is the only on-line EPR/radiation equipment in the USA, save for a pulsed-radiation/fast-EPR set-up built by Smaller at ANL which has been applied only to a small number of special radiation problems over the last few years, yet the laboratory does not have the in-house capability to pursue more recently developed applications.

Highly productive interactions between pulse radiolysis optical studies in liquids and EPR studies of irradiated single crystals or of frozen glasses and powders have led to significant advances in the understanding of radiation biophysics; and it is clear that correlations of pulse radiolysis findings with on-line EPR ofirradiated solutions would markedly accelerate this highly productive line of inquiry. New applications of radiochemical procedures and/or optical pulse radiolysis techniques to "bona fide" biochemical research (as opposed to radiochemical or radiobiological problems), especially those involving redox properties, electron transfers, and the like, including projects already underway in this program could be facilitated by the utilization of an on-line EPR/radiation facility.

Other departments at Brookhaven are also interested in utilizing such a capability. Radiochemists in BNL's Chemistry Department have been using fast and slow pulse radiolysis apparatus (with the Febetron and 2-MeV Van de Graaff, respectively) in conjunction with their more "classical" radiochemical

(See Continuation Sheet)

17. Expected Results in FY 1974: (Cont'd.)

studies, and during the past two years Bielski has begun to apply radiolysis procedures to the study of biochemical redox reactions, including enzymic ones; while Holroyd is also employing pulse radiolysis to study the same materials. An on-line EPR/radiation facility would add significant scope to this work.

For several years P. Levy of the Solid State Physics group has been interested in setting up an on-line EPR/radiation station at BNL's Dynamitron accelerator for the study of irradiated solids. Prior to the reduction in operating funds for the group, equipment for the EPR components was acquired including special magnets axially bored to permit unperturbed transport of the electron beam; and a beam pipe and "cave" compatible with EPR use have been provided. Several years ago Levy and Mattern expressed strong interest in collaboration with us on radiobiological and radiochemical studies using the proposed EPR facility at the Dynamitron, provided that we be responsible for the special microwave cavities and other equipment required for aqueous solution work. In the interim, however, the Physics Department budget has forced termination of the development work, Mattern has left BNL, and the incomplete EPR facility is in limbo.

During the past year informal exploratory meetings were held between members of BNL Chemistry and Medical Departments regarding possible collaboration in applying radiochemical and pulse radiolysis techniques to biomedical research in conjuction with EPR capability, especially flow systems for studying transient free radical intermediates. An on-line EPR/radiation facility would be of the greatest importance in supporting this proposed collaboration.

The facility would utilize present equipment and experimental rooms of the Physics Department and would require a full-time junior scientist plus an estimated $10,000 for initial installation. Although justified here in terms of radiation biophysics, non-radiation biochemistry, and the correlation of free radical mechanisms between the two, the facility would also be a uniquely powerful analytical tool in research on free radicals of pollutants, as is pointed out in the supplementary 189. (Borg)

A number of peptides of histidine and cysteine will be prepared by the dinitrophenylene bridging method, both to demonstrate the method and for the acquisition of several hormone analogs not otherwise obtainable. The dinitrophenylene bridging method will be extended to peptides of tyrosine if possible. It is planned to put into use the first system of enzymatic deprotection and to combine the attachment and deprotection work into a system of solid phase peptide synthesis which allows for preparation of hormone analogs entirely in aqueous solution at about physiological conditions of pH and temperature. (Schwartz, Walter)

(See Continuation Sheet)

Project Title: Mechanisms of Action of Free Radicals and Hormones RX-03-02-(c)

18. Expected Results in FY 1975:

Logical continuation of the projects described above is to be expected, many of which anticipate more than one year of work. Tissue studies and related clinical investigations remain a program objective. If the broad cellular and biochemical investigation of damage mechanisms in membranes proves fruitful, an expanded effort would follow including extension to additional classes of pollutants and possible evaluation of screening tests for long-term toxicity. If the conjoint EPR/radiation facility has not been initiated it would become a strong objective for the following year.

19. Description and Explanation of Major Materials, Equipment and Subcontract Items:

FY 1975 Capital Equipment:

Equipment in support of the BNL/EPR radiation facility is estimated at $10,000, and a microwave frequency measuring apparatus for microwave for Q-band EPR is estimated at $6,000.

20. Proposed Obligations for Related Construction Projects:

None

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory
Laboratory

RX-Biomedical & Environmental Research
Program

1. Contractor:
Associated Universities, Inc.

Contract No.:
AT(30-1)-16

Task No.:

2. Project Title:
Molecular and Cellular Radiobiology
Storage and Transfer of the Genetic Message

189 No.:
RX-248

3. Budget Activity No.:
RX-03-02-(d)

4. Date Prepared:
May 1973

5. Method of Reporting:
Scientific Meetings
BNL Monthly Letter to AEC
Scientific Journals

6. Working Location:
Brookhaven National Laboratory

7. Person in Charge:
L. D. Hamilton

Principal Investigator:
L. D. Hamilton

8. Project Term:
Continuing

From: To:

9. Man-Years:

Direct Man-Years	FY 1973	FY 1974	FY 1975
Scientific & Professional	2.0	2.0	2.0
Other	4.5	4.5	5.0
Guests & Res. Collaborators	---	---	---
Total	6.5	6.5	7.0

10. Costs (In Thousands of Dollars):

	FY 1973	FY 1974	FY 1975
[redacted] search Division	144	153	161
[redacted] spital Division	28	32	39
Research Costs	172	185	200
Equipment Obligations	0	5	29

11. Reactor Concept:

12. Materials:

13. Publications:

Kim, C. H., Jamuar, M. P., and Hamilton, L. D. Mechanism of transfer of delayed hypersensitivity to Trichinella spirilis: the effect of immune serum. J. Exp. Parasitol. (in press). 17344

Jamuar, M. P., Kim, C. W., and Hamilton, L. D. Fine structure study of lymphocytes from animals hypersensitized to Trichinella spirilis antigen. J. Exp. Parasitol. (in press). 17446

McVicar, J. W., Richmond, J. Y., Campbell, C. H., and Hamilton, L. D. Observations of cattle, goats and pigs after administration of synthetic interferon inducers and subsequent exposure to foot-and-mouth disease virus. Canadian J. Comparative Med. (in press). 17300

14. Scope:

A) 200 Word Summary:

The long-range objectives of this program remain: understanding how radiation and other genetic chemical hazards in the environment cause human disease, especially cancer. Efforts are aimed at establishing the various nucleic acid structures and their interaction among themselves and with environmental chemicals with the greatest possible accuracy. Approaches include: study of the 3-dimensional structure of natural double-stranded DNA, RNA's and of synthetic polynucleotides and of their complexes with other molecules such as histones and novel chemicals; study of the role of RNA-dependent DNA polymerase in proliferating normal, tumor, and immunologically stimulated cells; and study of the effects of synthetic polynucleotides on viruses.

If synthetic and biological double-stranded RNA's continue to be superior as inducers of interferon and immune responses in general, and at such dosage as to be therapeutically practical, they will merit testing against a wide variety of viral diseases in animals and man, and as promoters of the immune response after radiation injury. While it is clear that in the first instance such tests might be confined to established viral diseases, in the event that the synthetic and biological polynucleotides prove to have no harmful side effects, they would warrant trial in disease in man where virus etiology is suspected though not proven. This might offer possibilities in the treatment of several malignant diseases. Additional effort is needed to develop nucleoside analogs for polynucleotide synthesis and to prepare and evaluate biological polynucleotide complexes; such polynucleotides may be more potent.

B) Supplement to 200 Word Summary:

Since the only way in which environmental hazards--radiation and chemical pollutants--at low doses and low dose rates could be accurately assessed would be directly from a precise understanding of how they induce

(See Continuation Sheet)

14. Scope: (Cont'd.)

effects such as oncogenesis, the long-range objectives stated above were established. The import of work in many laboratories is to assign a clearer role to viruses and chemicals found in the environment, which, in addition to radiation, may be genetically damaging and often oncogenic. The environmental hazards for oncogenesis require a more general knowledge of the interaction of viruses, chemical agents, and radiation.

For the long-term objective of assessing environmental hazards, it is necessary to arrive at how the physical structure of informational macromolecules is affected by a wide variety of chemicals, viruses, and physical agents. Control of protein synthesis by nucleic acids continues to be the central target. Despite advances, it is likely to remain central at least until the structure of ribosomes and transfer RNA molecules, etc., and their interaction and control are elucidated. Despite world-wide expansion of research on this subject, further nucleic acid protein studies will be needed for decades before intracellular regulation in higher organisms is understood. As these factors are sorted out, there is a widely recognized need to integrate the interactions of all these environmentally damaging agents: viruses, chemicals and radiation.

Study of the interaction of chemicals with the informational macromolecules needs extension and entails expanded research on the macromolecules themselves and of the enzymes involved in their own synthesis and protein synthesis generally. These interactions are central to understanding how factors in the environment influence heredity and distort differentiation. They also relate to how viruses, chemicals and radiation induce somatic mutation, and how they and other factors accelerate aging.

The approach to a precise understanding of how radiation and chemical pollutants induce effects such as oncogenesis and genetic mutation is being made in this program in several diverse but complementary and directly interrelated ways: (1) In collaboration with the Biophysics Department and MRC Unit at King's College, London (with Professor M.H.F. Wilkins and Drs. W. Fuller and W.J. Pigram), the 3-dimensional structure of natural double-stranded RNA's and of synthetic polynucleotides and of their complexes with other molecules, such as histones and novel chemicals is studied. These complexes are studied not only because of their potential increase in the biological effectiveness of these molecules but also because of the light they might throw on the interaction and control of the informational macromolecules (for which the synthetic polynucleotides may be considered models). These studies should thus assist in defining the targets of environmental damage and help understand the possible synergistic effect of environmental chemicals in promoting this damage with radiation. (2) In collaboration with Dr. E. Popenoe (this Department), the role of RNA-dependent DNA polymerase is studied in proliferating normal, tumor, and immunologically stimulated cells. Using poly (rA·dT) as a primer it has been possible to

(See Continuation Sheet)

14. Scope: (Cont'd.)

show the presence of reverse transcriptases in HeLa cells, normal embryonic tissue, and transformed lymphocytes. The aim is to understand the biological significance of this activity. The immune system is a prime target of environmental damage. The study of the RNA-dependent DNA polymerase in lymphocytes after antigenic stimulation should serve not only as a model to understand how radiation and other environmental hazards might interfere with the workings of this system, but also as a model of how environmental damage might interfere with the regulation of cell division, leading to uncontrolled division. These studies complement others on how synthetic polynucleotides work and how they interact with histones--possible gene regulators. (3) The effects of synthetic polynucleotides on herpesviruses and other viruses are studied (see below). Several lines of evidence suggest that herpesviruses may cause cancer in the frog, chicken and man. The herpesvirus is associated with the Lucke carcinoma--a renal carcinoma of the frog Rana pipiens, and with Marek's disease--lymphoid tumor in chickens. Burkitt's lymphoma and infectious mononucleosis in man are associated with the herpesvirus--the Epstein-Barr virus--having some relation to herpes simplex and the virus from Lucke carcinoma. Finally herpes simplex type 2 virus has been found associated with cervical carcinoma in women. The studies on the effectiveness of synthetic polynucleotides against herpesviruses relate to their eradication as possible co-factors in oncogenesis. These studies also have the potential of being used to study possible synergism between irradiation and herpesviruses in oncogenesis, thus illuminating how radiation and viruses might interact to produce damage.

15. Relationship to Other Projects:

Related studies are carried on in laboratories throughout the world. At BNL Biology Department, these include Studier on genetics and physiology of bacteriophage T_7 DNA, Lacks on the mechanisms of bacterial transformation by DNA and genetic recombination, and Elkind on radiation damage to DNA.

Others working on structure of polynucleotides with x-ray diffraction include Alexander Rich at MIT and David Davies at NIH, Bethesda (structure of synthetic polynucleotides); Robert Langridge, Princeton (structure of ribosomes, tRNA and viral RNA); and V. Luzatti at Strasbourg, France (structure of nucleoproteins). It is believed there is no identical work being carried on in any AEC laboratory. The structure of an actinomycin-deoxyguanosine crystalline complex as a possible model for the stereochemistry of actinomycin binding to DNA is being studied by Sobell and Jain, Rochester.

Kornberg at Stanford, Bollum at Kansas, Gester at Columbia, Richardson at Harvard Medical School, and Khorana at MIT are studying the enzymic synthesis of DNA and synthetic deoxypolynucleotides. Dudock at State University of New York, Stony Brook, is determining the primary sequences

(See Continuation Sheet)

15. Relationship to Other Projects: (Cont'd.)

of transfer RNA's. Studies on protein synthesis are being made by Nirenberg, NIH, Bethesda; Lipmann and associates at Rockefeller University; and Ochoa and co-workers at New York University.

Watson at Harvard and Cold Springs Harbor; Jacob and Monod at Pasteur Institute; Fraenkel-Conrat and Singer, University of California, Berkeley; and Spiegelman at Institute for Cancer Research, Columbia University, are studying the transfer of information by messenger RNA, its relation to DNA and ribosomes and the interaction of various polynucleotides. Research on DNA-RNA hybrids is being done by Hayashi at La Jolla, Konrad at Berkeley, and Spiegelman; they have examined virus infected cells for such hybrids with conflicting results.

Baltimore at MIT and Temin at McArdle Laboratory for Cancer Research, Wisconsin, have found an RNA-dependent polymerase in virions of RNA tumor viruses. They, and now many others, are studying this problem, which has implications not only for oncogenesis by RNA viruses, but also for the general understanding of genetic transcription. Similar studies are being made by Spiegelman who has also described a DNA-directed DNA polymerase besides the RNA-dependent DNA polymerase activity possessed by oncogenic RNA viruses. This DNA-directed polymerase prefers double-stranded RNA as template and yields a principally double-stranded product. Gallo at NCI-NIH has described an RNA-dependent DNA polymerase in human acute leukemic cells, and Cavalieri has described how 5S and ribosomal RNA's can serve as primers for DNA synthesis using a DNA polymerase from E. coli. Crippa at the CNR Laboratory of Molecular Embryology, Arco Felice and Tocchini-Valentini at CNR Institute of Genetics and Biophysics, Naples, have described data in oocytes of Xenopus laevis consistent with the hypothesis that RNA-dependent synthesis is a way of amplifying ribosomal genes in the egg.

Other physico-chemical studies, e.g. temperature-jump, melting curves, etc., to determine sequence complementary on nucleic acids are made by Doty at Harvard and on transfer RNA's by Berg at Stanford. The Setlows at Oak Ridge National Laboratory are studying enzymes that repair DNA after damage, and Freifelder at Brandeis, the nature of the lethal lesion in DNA after radiation injury, genetic recombination. Increasing evidence from many laboratories is pin-pointing the nucleic acids as key targets of radiation damage as reported by the Setlows, K. Smith at Stanford, Howard-Flanders at Yale, M. Omerod at Chester Beatty, J. Lett at Colorado State.

The effects of polynucleotides and of other interferon-inducers, especially the anti-viral and anti-tumor effects are studied by Field, Tytell, Lampson, and Hilleman at the Merck Institute, Baron and Levy at NIH, Merigan at Stanford, and Chamberlain at Berkeley. Hillman, Baron, Levy,

(See Continuation Sheet)

15. Relationship to Other Projects: (Cont'd.)

Merigan, Colby at University of Connecticut, and Carter at Johns Hopkins University are working on interferon stimulation and factors affecting interferon. Braun at Rutgers University, and Johnson at University of Michigan are working on the adjuvant action of polynucleotides on humoral and cell-mediated immunity. Morrell at Upjohn Company, Kalamazoo and Talal at NIH, are studying the effects of polynucleotides on macrophages and on immunity and tolerance. Although some of these investigators work on closely related biological effects, the studies done here and at collaborating laboratories differ importantly in: (1) physical characterization of the polynucleotide complexes; (2) uniqueness of viruses studied, e.g. FMDV only at Plum Island; (3) uniqueness of experimental tumors tested at Sloan-Kettering; (4) comprehensiveness of pharmacology and toxicological studies at Sloan-Kettering; (5) broadness of spectrum of biological effects studied with same complexes.

16. Technical Progress in FY 1973:

During the past 12 months there has been an increase in thinking and experiment on the interaction of various molecules with nucleic acids and synthetic polynucleotides in collaboration with Drs. Fuller, M. Davies and Pigram, King's College. This has arisen as a reverse spin-off from efforts to decrease the biodegradability of synthetic polynucleotides in vivo by complexing them with other molecules and also because of increased interest in how environmental pollutants, e.g. polynuclear hydrocarbons, may damage by complexing with nucleic acids. Since intercalation--an interaction whereby the planar portion of a molecule slips in between adjacent base-pairs in double-helical DNA--is widely assumed to be an important mechnaism of the action of many basic drugs, e.g. acriflavin, ethidium, etc., but rigorous proof has been lacking hitherto and models have been ambiguous, the interaction of daunomycin with DNA was reviewed further.

Daunomycin--a glycosidic anthracycline antibiotic widely used for the palliation of acute leukemia and solid tumors in man--is a molecule of considerable biological interest. Its biological activity seems to be due to complex formation with DNA. Evidence for the first time from both x-ray diffraction and molecular model-building studies have provided data on the stereochemistry of intercalation into DNA of daunomycin. These are the first studies to present detailed x-ray evidence that intercalation of a chemical into DNA is occuring and to give accurately the degree of untwisting of the DNA molecule--an inevitable consequence of intercalation--per molecule of bound intercalated chemical. There are many references in the literature, particularly on circular DNA, to the paper by Fuller and Waring (Bunsengesellschaft für Physiklische Chemie, 68, 805, 1963), where a value for this important parameter--i.e. the untwisting of DNA--was proposed.

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

However, this work with ethidium bromide did not follow the change in layer-line spacing with increasing bound drug; the value was based on molecular model-building. Although two other papers have described x-ray diffraction studies of the intercalation of planar molecules with DNA, a key contribution from the studies reported here on the interaction of daunomycin with DNA was the location of the first zero in the equatorial Fourier transform, described for the first time in such interactions. This gave hard evidence on the molecular geometry of the complex. The value derived in these studies for the untwisting of the DNA helix per molecule drug bound is most important in studies on circular DNA and will become increasingly important in studies on the structure of the chromosome and how the chromosome functions.

All previous evidence suggesting that daunomycin intercalates into DNA was based on solution studies whose interpretation rests on analogies with similar studies on ethidium bromide and the acridines. For these molecules earlier x-ray work was taken as major evidence for intercalation; but, in fact, it was not nearly so unambiguous as the x-ray data reported in these studies on daunomycin and DNA. In sum this is a contribution to the stereochemistry of intercalation, widely assumed to be a key mechanism in the action of many basic drugs and a possibly important way in which polynuclear hydrocarbons, largely emitted by the burning of coal, interact with the informational macromolecules to induce such late effects as oncogenesis.

The single crystal analysis of the 1:2 complex of actinomycin with deoxyguanosine by Sobell and collaborators provides strong evidence for an intercalation model for the interaction of actinomycin with the DNA double helix. An earlier model proposed by Hamilton, Fuller, and Reich was based on specific hydrogen bonding between groups on the actinomycin with those on the guanosine residues in the DNA. One difficulty with the intercalation model is that the basis for the high specificity of the DNA-actinomycin interaction is not clear. It may be, therefore, that the external binding model represents a transient recognition state which is a preliminary to strong binding of the actinomycin through intercalation. In fact, for many molecules which bind to DNA, there appears to be an equilibrium between a strong intercalation binding site and a weak external binding site. Work here on daunomycin binding showed that the degree of intercalation varied with the relative humidity of the environment in which the fibers of the DNA daunomycin complex were maintained. The degree of intercalation increased with the increasing relative humidity presumably because the increase in the amount of water in the fiber environment tended to increase the hydrophobic contribution to the intercalation interaction. Experiments to date with DNA-actinomycin fibers have not yet given clear evidence for intercalation. However, these studies are being extended by maintaining fibers of the complex at very high humidity in an effort to favor the intercalation type of binding.

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

As part of the program of complexing polynucleotide complexes with other molecules, e.g. histones, to decrease their biodegradability in vivo and hence potentially increase their biological effectiveness, double-helical polynucleotide molecules were complexed with histones and with the basic polypeptides poly-L-lysine and poly-L-arginine. For some time it has been clear that nucleohistone in its native state is in a contracted form. One interpretation of x-ray diffraction studies of nucleohistone suggests that it has a supercoiled structure; electron-microscope studies on chromatin support the idea of such structures. But the x-ray data are not unequivocal; DNA complexed to histone is presumably in the B conformation and diffraction patterns are therefore not overly sharp or detailed. Moreover soluble preparations of such complexes needed for x-ray diffraction studies of fibers of nucleohistone are extremely difficult to prepare (see below). For these reasons, if superstructures were induced in synthetic polyribonucleotides which are in the A conformation (giving sharper and more detailed x-ray patterns), by complexing with simpler model molecules, e.g. basic polypeptides, x-ray diffraction studies of such model complexes could throw light on supercoiling in nucleohistones and the structure of chromosomes.

Complexing of double-helical polynucleotides with histones most frequently gives rise to an insoluble precipitated complex. Only occasionally, by adjusting the concentration of histones relative to polynucleotides, by complexing in high salt concentration, and then by gradual and careful dialysis of these complexes has it been possible to retain complexes that remained soluble at physiological salt concentration needed for the therapeutic trial and x-ray diffraction studies of such complexes. Soluble complexes have been obtained more reproducibly from complexing poly(rI·rC) with polylysine or polyarginine in a 10:1 ratio. Such complexes have shown significant increases in their melting temperature (TM) and also in resistance to ribonuclease digestion as compared with poly(rI·rC) alone. Preliminary x-ray patterns of fibers of complexes of poly(rI·rC) and polyarginine show signs of sharper reflections near the centers of the patterns and suggest that there is, in fact, superstructure in these synthetic RNA complexes. These studies continue.

In studies here and in collaboration with Dr. Chester Southam (Jefferson Medical College of Thomas Jefferson University, Philadelphia), the biological effects of various complexes of synthetic polynucleotides by themselves and complexed with other molecules have been tested against Semliki Forest virus (SFV) infection in mice and in human cells in culture. SFV is a mosquito-borne (RNA) virus (arbovirus group A) of the same antigenic group as Eastern, Western and Venezuelan Equine Encephalitis viruses (EEE, WEE, VEE). It causes lethal encephalitis in adult mice when inoculated intraperitoneally (ip) or intracerebrally (ic) and has a cytopathogenic effect (CPE) on human cells in culture. It can cause encephalitis in man

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

but does not have the high virulence of WEE, EEE, and VEE. SFV is therefore of interest as a potential cause of human disease, and as a relatively safe laboratory model for the virulent Equine encephalitis virus infections which are of immediate economic concern in veterinary medicine and a perennial threat to man.

The results of further experiments with polynucleotides against SFV infection in mice were in excellent agreement with earlier results. Mice which were treated with 100 μg of poly(rI•rC) or 100 μg poly(rA•rU) 2-4 hr before ip injection of SFV were usually protected, whether treatment was continued or not. Thus a single prophylactic injection of 100 μg poly(rI·rC) gave complete protection 0/35 deaths, a single injection of 10 μg gave 1/40 deaths. As noted previously, most mice given SFV ic died of the infection, but those protected lived slightly longer than those in untreated groups. Mice which survived as a result of poly(rI•rC) treatment were challenged with a second injection of SFV ip 6 weeks after the first dose to determine whether they had developed immunity as a result of the earlier exposure to the virus. In the two experiments completed so far, mice which had previously received SFV and poly (rI·rC) had a lower mortality than did control mice. These challenging data are being followed up. Complexes of poly(rI•rC) and histone and of poly(rI•rC) and polylysine or polyarginine had antiviral activity against SFV infection in mice but from the preliminary data it is not yet possible to say whether this was enhanced compared with the activity of poly(rI•rC) alone. These studies are continuing.

In collaboration with Drs. F. M. Schabel, H. E. Skipper and W. R. Laster (Kettering-Meyer Laboratories, Southern Research Institute, Birmingham, Alabama), poly(rI•rC) and poly(rA•rU) were tested as prophylactic agents against spontaneous AKR lymphoma in AKR mice. AKR mice were selected that were approximately 6 months of age, but free of grossly apparent clinical lymphoma at the time treatment was begun; i.e., animals had no grossly discernible enlarged thymus, spleen or peripheral lymph nodes. After treatment with poly(rI·rC) or poly(rA·rU), there was no significant change in the cumulative mortality pattern. There were no differences in the numbers of mice in control and treated groups dying without gross signs of disease. Treatment given before the development of frank clinical signs of leukemia or lymphoma in AKR mice can, in fact, abort the development of the disease. Thus in mice treated with palm 0-ara-C at 5 mg/kg, treated once a day for 7 days, then rested for 7 days, then treated for 7 days, then rested for 7 days, etc., from 6-12 months of age, treatment beginning at 180th day of life, there was about a 40% disease-free survivor rate, compared to about a 10% survivor rate in untreated control animals. The data collected so far fail to indicate that, at the doses and schedules used, either poly(rI•rC) or poly(rA•rU) had any influence on the subsequent development of clinically

(See Continuation Sheet)

16. Technical Progress in FY 1973: (Cont'd.)

recognizable and lethal leukemia-lymphoma in AKR mice. Consideration is being given to the possibility of beginning the treatment at an earlier age since evidence from other viruses indicates that polynucleotides are more effective when given prophylactically; also to more intensively sequential dosage schedules

To understand how polynucleotides and other interferon inducers work with a view to increasing their biological effectiveness, several in vitro approaches have been used. Preliminary experiments indicate that the addition of poly (rI·rC) to the antigen, bovine serum albumin (BSA) added to lymphocytes incubated in vitro from mice previously exposed to BSA antigen in vivo, significantly increased lymphocyte transformation compared to that after exposure to BSA alone. These studies are being extended. In cultures of HeLa S-3 cells, synchronized with amethopterin and adenosine followed by release of the block with thymidine, poly(rI·rC) and poly(rA·rU) significantly inhibited DNA synthesis in S phase cells (>30%) with no effect on protein and RNA synthesis. Poly(rI·rC) had no effect on DNA synthesis in log phase cells. This highly specific finding is being followed up; it is important not only for understanding the way in which synthetic polynucleotides induce their effects but may prove to be a significant probe to understanding further, the details of DNA synthesis.

The collaborative studies with Dr. E. A. Popenoe of this Department are reported in RX-03-02-(a).

17. Expected Results in FY 1974:

It is still hoped to complete papers on the high-resolution data and refinement of B DNA and on the complex of poly(dA·rU); as indicated previously, completion of these papers depends on the cooperation of research collaborators. The large paper on intercalation vs. external binding of drugs to DNA (in preparation over several years with Drs. Fuller and Pigram) needs some additional Fourier transform compilations including a program of calculation on the packing of molecules. It is hoped that this can be completed within the next 12 months. Further experimental work on the interaction of ethidium bromide and DNA will be carried out to obtain evidence, if possible, on the increase of layer-line spacing with increased bound drug analogous to the findings with daunomycin and DNA. Studies on the interaction of actinomycin and DNA will be extended by maintaining fibers of the complex at very high humidity in an effort to favor the intercalation type of binding and obtain clear evidence for intercalation in this complex.

Further x-ray diffraction studies will be made of complexes of poly(rI·rC) and poly(rA·rU) with polyarginine and polylysine to illuminate the structure of nucleohistone and hence of the chromosome. If the data from these model

(See Continuation Sheet)

17. Expected Results in FY 1974: (Cont'd.)

interactions warrants, they will be extended by fractionating histones and then studying the interaction of polynucleotides with arginine-rich and lysine-rich histone fractions.

The biological effectiveness of such complexes, characterized here by physico-chemical studies, including temperature-melting profile (TM) and resistance to ribonuclease, will be assayed against SFV infection in mice here and in collaboration with Dr. Southam. Also in collaboration with Dr. Southam the effects of polynucleotides will be tested topically against HSV and type 2 given intravaginally in mice. This produces an acute and chronic vaginitis, and the possible oncogenic role of this virus will be explored with and without polynucleotides and other possible enhancing or diminishing agents.

If opportunity permits, the U.S. Department of Agriculture, Veterinary Sciences Research Division, Denver, have indicated an interest in studying the effects of polynucleotide complexes prepared here against VEE virus infection in small animals. Similarly, when collaborators are ready, studies will be resumed on the effects of polynucleotide complexes on FMDV in large animals, e.g. cattle, goats and sheep, to include the effects of multiple rather than single doses of the complex, a comparison of routes of administration, etc. These studies will be carried out in the carrier state, i.e. in animals that have the virus without necessarily manifesting the disease clinically, in collaboration with Drs. M. Brugh and H. Bahnemann (Pan American Health Organization--WHO--Foot-and-Mouth Disease Center, Rio de Janeiro). The carrier-state animals in Rio have a considerable advantage over experimentally induced disease in large animals at Plum Island because of the considerable heterogeneity in the response of large animals infected experimentally. The carrier animals already have the virus; moreover the smaller size of Brazilian carrier cattle, and other animals, would conserve the use of synthetic complexes.

The studies aimed at elucidating the effects and mechanism of action of polynucleotides in vitro will be extended. These include the enhancement by polynucleotides of lymphocyte transformation in response to specific antigenic stimulation and the inhibition of DNA synthesis by polynucleotides in synchronized HeLa cells. Also, collaboration with Dr. E. Popenoe will continue on the biological significance of RNA-dependent DNA polymerase in HeLa and immune cells. These and other in vitro studies will aim toward understanding how polynucleotides and other interferon inducers work.

18. Expected Results in FY 1975:

Studies described above will continue with directions determined by the findings from current research.

(See Continuation Sheet)

19. Description and Explanation of Major Materials, Equipment and Subcontract Items:

FY 1975 Capital Equipment:

One-third share of the ultracentrifuge described in RX-03-02-(a) will cost $24,000.

20. Proposed Obligations for Related Construction Projects:

None

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory — Laboratory

RX-Biomedical & Environmental Research — Program

1. Contractor: Associated Universities, Inc.
Contract No.: AT(30-1)-16
Task No.:

2. Project Title:
Biological Studies of Materials Arising from Energy Operations--SUMMARY
189 No.: RX-377

3. Budget Activity No.: RX-03-06

4. Date Prepared: May 1973

5. Method of Reporting: See Sub-activities

6. Working Location: Brookhaven National Laboratory

PRIVACY ACT MATERIAL REMOVED

7. Person in Charge: See Sub-activities

Principal Investigator: See Sub-activities

8. Project Term: From: To: To be initiated in FY 1975

9. Man-Years:	FY 1973	FY 1974	FY 1975
Sci., Res. Assoc. (Ph.D. or Equiv.)			5.0
Visiting Sci.			---
Prof. (B.S. or Equiv.)			---
Sci. & Prof. - Total	---	---	5.0
Technical	---	---	7.5
Adm. & Clerical	---	---	---
Guests & Research Collaborators	---	---	---
Total	---	---	12.5

10. Costs (In Thousands of Dollars):	FY 1973	FY 1974	FY 1975
Labor (including benefits)			200
Mats., Trav., Dev. Subcont., Spec'l Proc.			36
Reactor, Accel., and/or Computer Usage			1
Allocated Technical Services			6
Gen. & Adm. Overhead			
Total Research Cost	0	0	
Equipment Obligations	0	0	82

11. Reactor Concept:

12. Materials:

RX-377

PRIVACY ACT MATERIAL REMOVED

1179320

Project Title: Biological Studies of Materials Arising from Energy Operations RX-03-06

SUMMARY

Sub-Activity	Title
RX-03-06-(a)	Free Radical Studies on Mechanisms of Action of Pollutants
RX-03-06-(b)	Interaction of Chemicals with Nucleic Acids
RX-03-06-(c)	The Effects of Pollutants on Cells Studied in Tissue Cultures

(See Continuation Sheet)

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory
Laboratory

RX-Biomedical & Environmental Research
Program

1. Contractor:
Associated Universities, Inc.

Contract No.: AT(30-1)-16

Task No.:

2. Project Title:
Biological Studies of Materials Arising from Energy Operations
Free Radical Studies on Mechanisms of Action of
Pollutants

189 No.: RX-379

3. Budget Activity No.:
RX-03-06-(a)

4. Date Prepared:
May 1973

5. Method of Reporting:
Scientific Meetings
BNL Monthly Letter to AEC
Scientific Journals

6. Working Location:
Brookhaven National Laboratory

7. Person in Charge:
D. C. Borg

Principal Investigator:
D. C. Borg

8. Project Term:
From: To:
To be initiated in FY 1975

9. Man-Years:

Direct Man-Years	FY 1973	FY 1974	FY 1975
Scientific & Professional	---	---	1.0
Other	---	---	2.5
Guests & Res. Collaborators	---	---	---
Total	---	---	3.5

10. Costs (In Thousands of Dollars):

	FY 1973	FY 1974	FY 1975
Research Division	0	0	88
Hospital Division	0	0	0
Research Costs	0	0	88
Equipment Obligations	0	0	60

11. Reactor Concept:

12. Materials:

Project Title: Biological Studies of Materials Arising from Energy Operations
Free Radical Studies on Mechanisms of Action of Pollutants RX-03-06-(a)

13. Publications:

Project not yet initiated.

14. Scope:

A) 200 Word Summary:

This proposal includes two major investigative projects: free radical studies of pollutant carcinogens and mutagens, and free radical studies of ozone and nitrogen oxide and of nitrate toxicity. Recently, evidence has been forthcoming that some chemical carcinogens act by combining with DNA by way of free radical reactions. These carcinogens include many of the atmospheric pollutants produced by the burning of fossil fuels for both electrical and automotive power (such as polycyclic hydrocarbons), and there are indications that some secondary products of pollution that are components of photochemical smogs (such as ozone and higher nitrogen oxides) may also be carcinogenic in a similar way. The immediately reactive ("proximate") forms of the carcinogens, following conversion by cellular metabolism, appear to be cationic free radicals or possible chemically related epoxides. The special EPR facilities developed at BNL are uniquely suited to monitor reactions of this kind, whose analysis should contribute significantly to a systematic understanding of those molecular properties of pollutants which lead to carcinogenesis or to mutagenesis (which appears to be a closely related process at the cellular level). A free radical test system monitored by EPR holds promise as a rapid, presumptive screening test for carcinogenicity among a group of hydrocarbons.

B) Supplement to 200 Word Summary:

The ongoing research at BNL (see RX-03-02-c) could readily be extended to include relevant pollutants. The responses of reactions involving radicals produced from pollutants in the presence of DNA constituents to different free radical generating systems (physical, chemical, and enzymatic) and to the inhibiting (protective) effects of antioxidants, free radical scavengers, etc., would be followed by EPR methods, which would also be assessed for their possible utility in assaying pollutant toxicity. However, the value of these free radical studies in evaluating the carcinogenic and mutagenic potential of pollutants could be amplified by coordinated investigations of three kinds: 1) biophysical measurements of the complexed product of carcinogen/mutagen attached to DNA or to its constituents, 2) kinetic analysis of the free radical combining reactions, and 3) evaluations of biological factors that could relate the reactions being studied more directly to possible toxicity in vivo. These related studies are discussed further:

1) Nuclear magnetic resonance (NMR) spectroscopy can be used to help reveal details of the attachment of carcinogens to specific sites. Although

(See Continuation Sheet)

14. Scope: (Cont'd.)

NMR cannot be applied to complex reactions as readily as can EPR, and it has much lower intrinsic sensitivity, it is capable of studying the final diamagnetic (i.e., nonparamagnetic) products of the reactions whose intermedicate combining steps would be analyzed by EPR methods.

2) The kinds of EPR techniques to be used in the investigations of reactive free radicals are capable of providing rich detail regarding the chemical nature and properties of paramagnetic reaction intermediates, but under the circumstances envisioned the accurate quantitative data required for kinetic analyses are not usually obtainable. However knowledge of reaction kinetics can be invaluable in making rigorous interpretations of reaction mechanisms, so the value of EPR studies of pollutants could be usefully supplemented by coordinating them with kinetic studies already under way or projected for inclusion in the present BNL research program studying the bioenergetics of free radicals.

One current collaboration with the Chemistry Department uses stopped-flow and temperature-jump methods to analyze certain free radical reactions. With the further provision of a senior technician or postdoctoral-level scientist, this collaboration could supplement studies of free radical reactions of pollutants with kinetic measurements.

Recently several research centers have recognized the great potential of using radiation methods to study certain kinds of biochemical reactions, especially oxidation-reduction reactions involving free radicals. Both radiation chemistry and pulse radiolysis approaches are applicable. At BNL a proposal is presently being developed to apply these radiation methods to biophysical problems, and a major part of the proposal concerns the development of an experimental facility for EPR measurements on-line at an existing electron accelerator (The Dynamitron), using special EPR components already on hand (Physics Department). Collaboration between Medical, Chemistry, and Physics Departments is proposed, and a supplementary budget of $55,000/yr (plus $10,000 installation costs for the first year) is being sought, but no funds have yet been committed. The present scientific justification is directed toward problems of radiation biophysics, non-radiation biochemistry, and the correlation of free radical mechanisms between the two. However, radiation is potentially a very useful means of generating reactive free radical forms of pollutants, and pulse radiolysis could be used to obtain important kinetic data, while the on-line radiation/EPR facility would be a uniquely powerful analytical tool in research on free radicals of pollutants.

3) It is now recognized that the proximate forms of carcinogens and mutagens (the forms that actually react at biological target sites) may be significantly altered by metabolic biochemistry from those to which the

(See Continuation Sheet)

14. Scope: (Cont'd.)

organism is initially exposed. Hence the biological significance of EPR findings regarding the potential toxicity of pollutants would be heightened if host-mediated assays were employed, using appropriate implantations of agents in animals to permit biotransformations (whether activating or detoxifying) to take place. EPR surveys of implants or host tissues themselves would allow the possibility of developing an in situ or in vivo EPR assay for pollutant carcinogenicity or mutagenicity to be evaluated.

Nitrogen oxides are among the volatile products of combustion of fossil fuels (as well as of tobacco smoke), and their atmospheric photochemistry is responsible for most of the color and much of the recognized biological irritation due to photochemical smogs. As oxidizing chemicals, some even being free radicals (e.g., nitric oxide), radiomimetic character might be anticipated, possibly including long-range effects such as carcinogenesis or mutagenesis. However, little documentation is in hand regarding the distribution, reactions and biological fate of nitrogen oxides or the other atmospheric oxidants associated with fuel combustion, such as ozone.

EPR and related double resonance spectroscopies have the potential to analyze from biological samples (including cells and tissues) the paramagnetic centers that are formed by the association of nitrogen oxides with hemoglobin and with other heme proteins (especially cytochromes), as well as more stable free radicals (such as lipoperoxides) that may be produced by the reactions of radiation, nitric oxiden, ozone, etc., with lipids and biological membranes. In fact a crude EPR survey some years ago reported the generation of paramagnetic sites in distant tissues of animals that breathed air containing various combustion products, including nitrogen oxides.

The special capability of the BNL bioenergetic program involving free radicals to carry out EPR and double resonance measurements on tissue samples could readily be extended to monitor the attack of ozone and nitrogen oxides in intact animals. Additional studies on cells, their components, and on biochemicals would complement the work, and its interpretation would be further supported by accompanying microscopic and biochemical analyses. The possibility should be considered of using EPR techniques to develop an assay for human exposure to some of the environmental agents of this class.

[redacted]rate buildup in ground water may result from leaching that follows the strip mining of coal, as well as from sewage and fertilizers. The biological consequences have been thought to be mostly those associated with eutrophication; however, there are some indications that levels of both nitrites and nitrates in drinking water may be important to health.

(See Continuation Sheet)

Project Title: Biological Studies of Materials Arising from Energy Operations
Free Radical Studies on Mechanisms of Action of Pollutants RX-03-06-(a)

14. Scope: (Cont'd.)

Nitrites, which can be formed from nitrates by soil bacteria, may be converted by stomach acids to nitrosamines, some of which can be highly mutagenic. In addition, EPR signals of presently unknown significance have been found in tissues and cells exposed to relatively high levels of nitrite and/or nitrate in their water supplies. Although once again this suggests the possibility of an EPR assay of "effective" biological exposure, it may also have functional consequences in terms of cancer induction by other chemicals. For example, high levels of dietary nitrate enhance EPR signal amplitude but decrease tumor incidence (i.e., a protective effect) when given to animals exposed to various carcinogens. Clearly these preliminary findings should be followed up, and the EPR capabilities at BNL are well suited to this work.

15. Relationship to Other Projects:

NMR studies are planned in the Biology Department. Radiation techniques to provide kinetic information and further identification of reaction intermediates are in progress in the Chemistry Department (pulse radiolysis) and in the Physics Department (on-line EPR at the Dynamitron electron accelerator).

16. Technical Progress in FY 1973:

Project not yet initiated.

17. Expected Results in FY 1974:

Project to be initiated in FY 1975.

18. Expected Results in FY 1975:

Present programs may be extended to include consideration of non-nuclear pollutants with minimum additional support. Optimum implementation of the research activities noted would require an estimated addition of two scientific staff members and two technicians (plus one scientist and one technician associated with the radiation /EPR collaborative project). Stopped-flow and temperature-jump analyses of free radical reactions would require another technician.

The reaction of several classes of known carcinogens (aminonitro-quinolines, benzanthracene-benzpyrene, nitrosamines) with DNA constituents (bases, nucleosides, nucleotides, oligonucleotides) will be examined by EPR to determine whether or not combination occurs in the same way that radiation-produced free radicals combine.

(See Continuation Sheet)

18. Expected Results in FY 1975: (Cont'd.)

If evidence is found that combination is by means of free radicals, the reactions will be studied by stopped-flow techniques to obtain preliminary data on the kinetics of reaction, and electrochemical measurements (cyclic voltammetry, potentiometry) will be used to delineate oxidative reaction paths and potentials in different solvents.

The setting-up of an "on-line" EPR spectroscope at the Dynamitron, and installation of ancillary experimental apparatus would begin in collaboration with the Chemistry and Physics Departments.

19. Description and Explanation of Major Materials, Equipment and Subcontract Items:

FY 1975 Capital Equipment:

Additional equipment for full implementation: EPR spectrometer with Q-band EPR bridge ($60,000).

20. Proposed Obligations for Related Construction Projects:

None

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory
Laboratory

RX-Biomedical & Environmental Research
Program

1. Contractor:
Associated Universities, Inc.

Contract No.: AT(30-1)-16

Task No.:

2. Project Title:
Biological Studies of Materials Arising from Energy Operations
Interaction of Chemicals with Nucleic Acids

189 No.: RX-385

3. Budget Activity No.:
RX-03-06-(b)

4. Date Prepared:
May 1973

5. Method of Reporting:
Scientific Meetings
BNL Monthly Letter to AEC
Scientific Journals

6. Working Location:
Brookhaven National Laboratory

7. Person in Charge:
L. D. Hamilton

Principal Investigator:
L. D. Hamilton

8. Project Term:
From: To be initiated in FY 1975
To:

9. Man-Years:

Direct Man-Years	FY 1973	FY 1974	FY 1975
Scientific & Professional	---	---	2.0
Other	---	---	2.5
Guests & Res. Collaborators	---	---	---
Total	---	---	4.5

10. Costs (In Thousands of Dollars):

	FY 1973	FY 1974	FY 1975
[redacted] Research Division	0	0	119
Hospital Division	0	0	0
Research Costs	0	0	119
Equipment Obligations	0	0	7

11. Reactor Concept:

12. Materials:

Project Title: Biological Studies of Materials Arising from Energy Operations
Interaction of Chemicals with Nucleic Acids RX-03-06-(b)

13. Publications:

Project not yet initiated.

14. Scope:

A) 200 Word Summary

One of the long-range effects of non-nuclear energy systems urgently needing analysis is carcinogenesis (oncogenesis). This involves more than the direct effect in present generations since there is the possibility of genetic damage. Chemical carcinogens given at low doses over long periods of time present the same problem as do low doses of radiation at low dose rates: one has to extrapolate experience from high to low doses. Since the hazard from low doses of common pollutants generated by non-nuclear energy sources would only be deducible from a precise understanding of how these pollutants induce effects, studies are needed on how such environmental contaminants interact with the informational macromolecules, that is to say, nucleic acids. This approach offers the opportunity not only of understanding how toxic chemicals induce biological damage, but may also indicate ways to minimize damage. As knowledge of these interactions increases, it may serve as a rapid detection system for chemicals likely to cause genetic damage and tumors.

The aim of this study is prediction, not description--to tell before the fact what kinds of molecule are likely to cause intense pharmacological effects, notably oncogenesis and genetic effects.

B) Supplement to 200 Word Summary:

A survey will be made of soluble carcinogens (soluble in physiological solution) apart from polynuclear hydrocarbons--emitted from fossil-fuel power generating plants, and of soluble carcinogens--apart from polynuclear heterocyclic compounds and polynuclear aromatic hydrocarbons--present in urban atmosphere. Chemicals recognized as directly carcinogenic will be complexed with DNA, double-stranded RNA, and synthetic RNA so as to detect their exact points of interaction. The interaction of the carcinogens in highly specific ways with the DNA and RNA will be followed spectrophotometrically, by melting-curve analyses, and, when indicated by ultracentrifugation analyses. In critical cases, x-ray diffraction studies will be made of fibers of complexes and the data from these combined with molecular model-building to provide rigorous proof on the stereochemistry of the interaction of the chemical with the nucleic acid.

15. Relationship to Other Projects:

The proposed program extends to environmental pollutant studies on the interaction of drugs with nucleic acids carried out under budget activity

(See Continuation Sheet)

15. Relationship to Other Projects: (Cont'd.)

RX-03-02-d in collaboration with the Medical Research Council Biophysics Research Unit and Department of Biophysics, King's College, London, as part of a program on the storage and transfer of the genetic message.

Others working on the interaction of chemicals with nucleic acids include David Davies at the National Institutes of Health, Bethesda, and H. M. Sobel and S. C. Jane at Departments of Chemistry and Radiation Biology and Bio-Physics, University of Rochester. Several other laboratories are using Nuclear Magnetic Resonance (NMR) to discern the interaction between chemicals and monomeric substituents of nucleic acids. The proposed work will complement proposed studies of D. Borg in the BNL Medical Department on the interaction of chemicals with substituents of nucleic acids and eventually with nucleic acids themselves, to be followed by Electon Paramagnetic Resonance (EPR) spectroscopy and NMR.

These studies also relate to studies on research in mutagenesis being carried out by F. J. de Serres at Oak Ridge National Laboratory; on the relation of cancer induction and genetic damage by J. H. Weisberger and G. M. Williams at the National Cancer Institute, NIH, Bethesda; and extrapolating results of toxicity studies in laboratory animals to man by D. P. Rall, National Institute of Environmental Health Sciences, North Carolina.

16. Technical Progress in FY 1973:

Project not yet initiated.

17. Expected Results in FY 1974:

Project to be initiated in FY 1975.

18. Expected Results in FY 1975:

The first task will be a survey of carcinogens soluble in body fluids as well as polynuclear hydrocarbons that will require solubilization emitted from fossil-fuel power plants and also present in urban atmosphere. Pure compounds of chemicals recognized as directly carcinogenic will be complexed in vitro with DNA, various types of RNA, and synthetic polyribonucleotides. The interaction of the carcinogens with nucleic acids will be followed spectrophotometrically, by melting-curve analysis, and when indicated, by ultracentrifugation analysis. In addition, where it is possible to get complexes that remain re-soluble, x-ray diffraction studies will be made on fibers of the complexes, and the data from these combined with molecular model-building of the nucleic acids and the interacting molecules to determine the structure of the three-dimensional interaction of the complex. It

(See Continuation Sheet)

18. Expected Results in FY 1975: (Cont'd.)

is expected that the survey of classes of compounds directly interacting with DNA and other nucleic acids and their availability as pure compounds will be completed in the first year. In addition, a number of these interactions will have been studied in vitro by various physico-chemical techniques. The determination of the molecular interaction by x-ray diffraction and model-building is a more time consuming process, and at most, several complexes may be determined in a year.

19. Description and Explanation of Major Materials, Equipment, and Subcontract Items:

None

20. Proposed Obligations for Related Construction Projects:

None

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory Laboratory	RX-Biomedical & Environmental Research Program

1. Contractor: Associated Universities, Inc.
   Contract No.: AT(30-1)-16
   Task No.:

2. Project Title: Biological Studies of Materials Arising from Energy Operations
   The Effects of Pollutants on Cells Studied in Tissue Cultures
   189 No.: RX-389

3. Budget Activity No.: RX-03-06-(c)

4. Date Prepared: May 1973

5. Method of Reporting:
   Scientific Meetings
   BNL Monthly Letter to AEC
   Scientific Journals

6. Working Location:
   Brookhaven National Laboratory

7. Person in Charge:
   E. P. Cronkite

   Principal Investigator:
   E. P. Cronkite
   M. A. Bender
   A. L. Carsten

8. Project Term:
   From: To be initiated in FY 1975
   To:

9. Man-Years:

Direct Man-Years	FY 1973	FY 1974	FY 1975
Scientific & Professional	---	---	2.0
Other	---	---	2.5
Guests & Res. Collaborators	---	---	---
Total	---	---	4.5

10. Costs (In Thousands of Dollars):

	FY 1973	FY 1974	FY 1975
[redacted]esearch Division	0	0	133
[redacted]apital Division	0	0	0
Research Costs	0	0	133
Equipment Obligations	0	0	15

11. Reactor Concept:

12. Materials:

Project Title: Biological Studies of Materials Arising from Energy Operations
The Effects of Pollutants on Cells Studied in Tissue Cultures RX-03-06-(c)

13. Publications:

Project not yet initiated.

14. Scope:

A) 200 Word Summary:

The incidence of chronic pulmonary disease is increasing. Pollutants in the environment, cigarettes, and industrial dusts are suspected as the primary causes. Evaluation of suspected hazards by exposure of animals in closed, monitored environments would take decades and be extremely costly in manpower as well as dollars. Using a 3 tier tissue culture system, it is proposed to study the interactions of pollutants with pulmonary macrophages and neutrophils and to detect those pollutants with deleterious effects on human pulmonary epithelium. In addition, the effects of noxious particulate and gaseous pollutants will be studied in classical tissue culture systems. The pollutants shown to have deleterious effects in culture will be evaluated in animals.

B) Supplement to 200 Word Summary:

In the highly industrialized western society, man is subject to a changing incidence of genetic, cardiovascular, pulmonary, neurological and mental diseases and cancer. The incidence of pulmonary diseases is increasing but the suspected role of the environment in causing this increase has been inadequately evaluated. One must consider the following as potentially causative factors: the effect of radiation from all sources (the practice of medicine, nuclear power plants, industrial operations, etc.); the effect of processed foods in the diet; the effect of drugs used in the practice of medicine; the effects of chemicals used in industry; effects of industrial gas and liquid effluents contaminated by heavy metals; the carcinogenic effect of hydrocarbons; the general toxic effect of oxides of nitrogen and sulfur exhausted from fossil fuel plants; the effect of inner-city crowding on the spread of disease generally; and the effect of stress due to such influences on the individual as the uncertainties connected with modern national and internationa competition. None of these factors can be satisfactorily considered separately Each must be analyzed in relation to the others, the varying interactions thoroughly evaluated.

[redacted] studies comparable to those used to assess the hazard of inhaled plu[redacted] and uranium are required to identify the chemical and physical cha[redacted]istics of hazardous pollutants, the long-term animal studies will requ[redacted]1-2 decades. A method enabling more rapid answers would be useful in designing the long-term experiments. Thus, the initial proposal is to study the effect of environmental contaminants upon cell proliferation and function, including in vitro carcinogenesis, with diverse standard tissue culture systems. These would include in vitro cultivation of bone marrow

(See Continuation Sheet)

14. Scope: (Cont'd.)

cells, human alveolar macrophages, embryonic human tissue cultures, and lymphocyte cultures. These tissue cultures would be measured for: 1) doubling time, 2) growth fraction, 3) time parameters of the cell cycle utilizing the flow of tritiated thymidine labeled cells through mitosis, 4) the possible induction of somatic mutations (enzymatic changes), 5) changes in function, e.g., effects on production of erythropoietin and other enzymes by cultures of human renal glomeruli, and 6) cytogenic effects.

One theory on the development of emphysema suggests that a result of pulmonary irritants is the interstitial death of neutrophils and/or macrophages and the concomitant release of potent destructive lysosomal enzymes that produce microscopic foci of necrosis which, over a prolonged period, produce emphysema (Janoff). However, the detection of such an effect is almost impossible by ordinary serial section of lungs because the sizes of the lesions are too small. It is therefore proposed that a tissue culture system be developed to assay such effects. The system would consist of three layers of different cells in soft agar: the deepest layer consisting of an appropriate feeder system; the next layer consisting of human pulmonary epithelium obtained from viable fetuses; and when this culture is established, a third layer consisting of human alveolar macrophages and neutrophils exposed to particulate atmospheric and/or gaseous pollutants. The basis for this system is as follows: if the phagocytes ingest particles for which the cell is not programmed to inactivate, there is a high probability that the result would be the death of the neutrophil or macrophage and the release of the lysosomal enzymes (hydrolytic and oxidative) thus leading to the death of underlying pulmonary epithelial cells. In principle, this could be identified by the development of transparent plaques.

If the tissue culture studies produce positive results, chronic inhalation studies would be initiated using animals exposed to variable concentrations of the same materials used in the tissue culture studies. Lifetime observation of these animals would include: 1) the incidence of pulmonary disease, 2) somatic effects, 3) pulmonary function, 4) reproductive capability, 5) the induction of dominant lethals, 6) the effects on embryo in culture, and 7) carcinogenesis.

The proposed studies would be in collaboration with the BNL Department of Applied Science where pollutants would be prepared for use in tissue cultures and in animal studies. Dr. Michael Bender will participate for the cytogenetic studies, and a scientist will be required for the study of the effects on embryos.

(See Continuation Sheet)

Project Title: Biological Studies of Materials Arising from Energy Operations
The Effects of Pollutants on Cells Studied in Tissue Cultures RX-03-06-(c)

15. Relationship to Other Projects:

Related studies on pulmonary function, pulmonary surfactants, and elasticity are proposed by the BNL Department of Applied Science.

The experiments would lean heavily upon existing tissue culture techniques for the study of hemopoietic stem cell proliferation and differentiation and studies on renal glomerular cultures and studies on the induction of dominant lethals by tritium (RX-03-02-b and RX-01-01-c). Dr. Aaron Janoff, State University of New York at Stony Brook, is actively pursuing studies aimed at confirming his hypothesis on the release of lysosomal enzymes from neutrophils and macrophages as an etiologic factor in the production of emphysema and other chronic pulmonary disease. Contemporary studies on α_1 antitrypsin are closely related since individuals with congenitally determined pulmonary disease have an absence of or marked reduction in α_1 antitrypsin.

16. Technical Progress in FY 1973:

Project not yet initiated.

17. Expected Results in FY 1974:

Project to be initiated in FY 1975.

18. Expected Results in FY 1975:

Design studies will center on the development of environmental incubators for tissue culture techniques and preliminary studies will be made on producing plaques in the three layer tissue culture proposal for detection of pollutants harmful to the lung. With the appointment of additional scientists and technicians, tissue cultures will be established and control studies initiated to see the effect of gaseous pollutants (oxides of nitrogen and sulfur) upon the proliferation rates and the development of cytogenetic abnormalities. Incubators within which one can control the concentration of ambient gases will be designed and fabricated. Tissue culture flasks and dishes will be modified in order to insure a constancy of the concentration of the gases overlying the culture. The three tier culture technique to assay particulate pollutants will be established. When concentration of gases harmful to tissue culture are established and hazardous particulates are identified in the tissue culture system, facilities for long-term animal studies will be designed and fabricated.

(See Continuation Sheet)

Project Title: Biological Studies of Materials Arising from Energy Operations The Effects of Pollutants on Cells Studied in Tissue Cultures RX-03-06-(c)

19. Description and Explanation of Major Materials, Equipment and Subcontract Items:

FY 1975 Capital Equipment:

The purchase and conversion of incubators to control ambient gases and permit introduction of aerosols in equilibrium with the surface of tissue cultures is estimated to cost $15,000. The fabrication of specially designed long-term animal facilities will be a major expense in the following fiscal year.

20. Proposed Obligations for Related Construction Projects:

None