PROPOSAL AND AUTHORIZATION
FOR RESEARCH OR DEVELOPMENT

725504

HYO-149

1. Project Title: Medical Research			2. Date: May, 1959
3. Budget Activity No. Summary 6120, 6310, 6320	4. Budget Item No:	5. Contractor's No.	6. Method and Time of Reporting Progress: Quarterly & Special Reports
7. Contractor: Associated Universities, Inc. Brookhaven National Laboratory		8. Working Location: Upton, New York	9. Contract No. AT-30-2-GEN-16
10. Persons in Charge: L. E. Farr, M.D.		11. Starting Date: Continuing	

SUMMARY

The Medical Research Center

Brookhaven National Laboratory

Upton, L. I., New York

Project Title: Medical Research - 6120

17. Operating Costs (in thousands of dollars)	Estimated 1959	Estimated 1960	Estimated 1961
Labor (including benefits)	612	674	743
Materials, Travel, etc.	181	209	215
Development Subcontracts, Special Proc.	24	19	18
Total Direct	817	902	976
Special Power	-0-	-0-	-0-
Reactor and/or Accelerator Usage	46	54	50
Technical Services (from BNL Service Units)	104	98	104
General & Administrative Overhead	428	436	420
Total	1,395	1,490	1,550

18. Plant & Equipment Directly Required
(Shown here for information only)

	Estimated 1959	Estimated 1960	Estimated 1961
(A) Construction			
(B) Equipment (in thousands of dollars)	90	90	90

19. Direct Man Power	Estimated 1959	Estimated 1960	Estimated 1961
No. of Man Years			
Scientists, Research Associates	22.0	22.0	23.0
Visiting Scientists	6.0	6.5	6.5
Scientists - Total	28.0	28.5	29.5
Technical	56.5	57.0	59.5
Administrative & Service	9.0	10.0	9.5
Total	93.5	95.5	98.5

20. Comments

PROPOSAL AND AUTHORIZATION
FOR RESEARCH OR DEVELOPMENT

NYO-149

1. Project Title: Medical Research			2. Date: May, 1959
3. Budget Activity No. Summary 6120, 6310,6320	4. Budget Item No.	5. Contractor's No.	6. Method and Time of Reporting Progress: Quarterly & Special Report
7. Contractor: Associated Universities, Inc. Brookhaven National Laboratory		8. Working Location: Upton, New York	9. Contract No. AT-30-2-GEN-16
10. Persons in Charge: L. E. Farr, M.D.		11. Starting Date: Continuing	

SUMMARY

The research program of the Medical Department concerns itself with the biological effects of radiation and in particular with particle radiation of very short range or situations wherein the energy release is over a very short track. The researches thus are, and must be concerned inherently with studies of precise isotope localization, kinetics of distribution and redistribution, metabolism of organic compounds, functions of inorganic compounds, and the effects of excited atoms on the stability of large molecules or complexes. Advantage is sought of special situations wherein specific products or devices are concerned which may be applicable to medical therapy. Diagnostic studies in the widest sense are carried out on suitable disease states under study in the hospital. Such studies are concerned primarily with elucidation of the nature of the disturbance and the proper selection of individuals in a general population for a uniform response rather than with specific diagnostic routines for use in a large general medical clinic, although the latter is kept in mind.

The over-all scope of the medical program will not change through any budget year; however, in any given year the emphasis will be in those portions of the program for which unusual capabilities exist in the staff at that time or for which most promising leads have been developed. Since the work is part of the Department's continuing program, break-throughs will be exploited when warranted with imagination and determination. In other instances, the knowledge will be brought to the attention of collaborators or others for further development elsewhere.

Specifically, the program contains several component parts which relate the general statement as given above to the several fields of medicine. The continuing intensive exploration of particle radiation of short range has brought out the necessity for further dosimetric and instrumental developments. In the application of a therapeutic procedure such as neutron capture therapy, it becomes important to know the effect of a single fission event on a single cell so that the probability of biological effect can be estimated by a summation of knowledge of the probability of the capture reaction occurring together with the probable atomic distribution of the boron in the tissue of interest. Thus, it becomes important to know dose effects in terms not of tissue volumes but of cell population of varying types, closely intermingled, and for the larger part of their metabolism, carrying on identical biochemical reactions. Hence, from time to time greater emphasis must be placed upon differing particle bombardments ranging from cosmic rays, through boron-10 neutron capture with slow neutrons, to neutron and charged particle bombardment itself.

To elucidate effects, an intimate knowledge must be obtained of cell, organ, or tissue, and of entire mammalian metabolism so that not only is the specific reaction known and identifiable, but its relation and interrelationship to a whole host of other reactions is clearly known. While this is necessary to assess effects, other knowledge, together with a precise statement of the laws governing passage across cell membranes, must be sought out and established that specific isotopes may be placed in specific situations that specific effects may be observed. To the general area of knowledge concerned with such target placement, we have applied the term "selective kinetics".

(SEE CONTINUATION SHEET)

It is clear that capacity to place and fix an atom on a specific cellular target without diversion, wandering, or delay implies a concise knowledge which in the reverse could be employed to remove an atom from a cell, organ or tissue effectively and expeditiously and without engendering harm to the body as a whole or its constituent parts. While much work will be concerned with specific body constituents added under experimental conditions, it is clear that additional useful, extremely accurate, and rapid analytical methods are necessary. In part they will be pioneered through the further adaptation of machines, devices, and products of nuclear physics to the solution of specific biological, analytical problems. The exploration and development of activation analysis is an example of this effort.

By agreement with, and at the request of the Division of Biology and Medicine, the medical research program now in existence covers the following broad lines of investigation, all of which are closely integrated operationally.

Neutron Capture Therapy

Maps of Metal Pathways with Especial Reference to Trace Metals and to Central Nervous System Diseases.

Radioactive Elements in Organs and Tissues of Man

Radioactive Isotopically Labeled Cells for Predictions of Life Span, Functions and Progeny

Hematology of Radiation

Radiation Effects of Immunity and Allergy

Vitamin and Amino Acid Metabolism in Neoplasia and Normality

Labeled Proteins for Metabolic Observation in Cancer Evaluation

Radioisotopic Tracing of Total and Intermediate Carbohydrate Metabolism

Radioisotopes for Study of Protein and Nitrogen Metabolism

Selective Single Elemental and Colligative Activation

Clinical Management of Radiation Injury

Radioisotopic Labeling of Hormones to Determine Action Sites

Special Projects

The last notation, under the heading of "Special Projects," gives details of certain self-contained projects carried out by the Department, such as the operation of an occupational medicine clinic, educational conference, and the continuing medical study of the Marshall Islanders. While very important and significant in their own right, they derive from staff competence gained by Brookhaven experience rather than basically providing that experience for the scientists concerned. Costs for operating the occupational medical clinic are included in the general and administrative costs of the Laboratory and are distributed as Indirect Expense. On the other hand, costs for such Special Projects as the Marshallese studies are included in the costs of Radiation Effects with the major expense being borne by AEC Activity 6120.

The program of the Medical Department can be divided into two components, (1) which may be called the intramural program which is carried out by the regular full time staff at Brookhaven and, (2) the extramural program which is a joint venture between the regular full time staff at Brookhaven and a non-salaried research collaborator staff working part-time intermittently at Brookhaven but carrying on much of their research at their home institutions. Included in the extramural program are the educational activities of the Department. These range from lectures given to institutions and societies to an annual conclave in which attendence is by invitation only and one person from a specific discipline in each medical school of the United States and Canada is invited. This endeavor particularly well emphasizes the point that in publicizing its research

(See Continuation Sheet)

the Department inextricably blends its investigative work into its educational program. The conclaves cover specific, clinical, financial, and administrative phases of this new area of medical science on which the Department can offer guidance and counsel.

In 1952 the definitive plan for the ultimate staffing of the Medical Department was presented and approved in principle. Since that time additions to the staff have been made to fulfillment of that plan. In 1952 there were four scientists on the Senior Staff. By June 30, 1957, the Senior Staff had increased to 15 and during fiscal 1958, to 16. In 1952 the total scientific staff was 19, at the end of fiscal year 1957, it was 34, and by the end of fiscal 1959, it is expected to be 44. The increase in staff to final size should be as rapid as possible that the potentialities of the new medical center may be promptly realized. The additional facilities provided by the new building lend significant energy to the provisions of technical assistance and equipment required to maintain the momentum achieved in the research program. Realistic estimates of capital equipment are constantly reviewed so that items considered most useful in the program are available as the research requires.

The Medical Department has now developed its program in outline. During 1960 there should logically be an increase in intensity of work on most of the basic division of investigation particularly the following: (1) Neutron capture therapy and RBE studies of heavy particles; (2) the study of kinetics of distribution of metals as pioneered by work in Mn^{56}; (3) the application of mathematics to description of kinetics of distribution for further development of tracer theory with its manifold immediate applications such as capacity to alter fixation or to remove fixed isotopes; (4) the usefulness of short-lived isotopes in gaining a better understanding of cancer therapy; (5) the effects of radiation as a carcinogenic and mutagenic agent with particular reference to the usefulness of whole body counting techniques of determining whole-body burdens and their relation to fallout; (6) the effects of radiation on hematopoietic tissue and methods for prevention or amelioration; (7) intensive study of specific metabolic reactions both for placement of radioactive isotopes and for understanding and control of mechanisms involved therein; (8) the effects of radiation on production of anitbodies and allergies.

This list could be extended usefully, but it may be more desirable to summarize the effort to two main areas; (1) the biological effects and medical implication of radiation exposure; (2) the effect of development of reactors on medical concepts of their use and hazards. The planned increase in staff year by year to a total of 49 scientists is to augment and intensify the effort in these areas and to fill in the important segments of the stated program. The importance of the first is currently exemplified by the present controversy on the effects of fallout. One or two suitable whole-body counters, a miltiple-foci chrono-intensity detector, and additional animal facilities will enable the increased staff productively to work on these problems. The importance of the problem regarding reactors is pointed up by the number of reactors built, building or planned for the United States and the world. Some additional instrumentation and operating personnel again could profitably build on the present solid foundation. The cost increment while significant in terms of present budget is very small in terms of expenditures making these studies of importance.

Project Title; Medical Research - Summary (6120 - 6310 - 6320)

17. Operating Costs (in thousands of dollars)	Estimated 1959	Estimated 1960	Estimated 1961
Labor (including benefits)	1,073	1,319	1,531
Materials, Travel, etc.	283	368	400
Development Subcontracts, Special Proc.	36	28	28
Total Direct	1,392	1,715	1,959
Special Power	-0-	-0-	-0-
Reactor and/or Accelerator Usage	80	108	108
Technical Services (from BNL Service Units)	173	184	207
General & Administrative Overhead	730	833	846
Total	2,375	2,840	3.120

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18. Plant & Equipment Directly Required (Shown here for information only)	Estimated 1959	Estimated 1960	Estimated 1961
(A) Construction			
(B) Equipment (in thousands of dollars)	170	195	180

19. Direct Man Power	Estimated 1959	Estimated 1960	Estimated 1961
No. of Man Years			
Scientists, Research Associates	38.0	43.0	48.0
Visiting Scientists	9.0	9.5	9.5
Scientists - Total	47.0	52.5	57.5
Technical	103.5	115.5	133.5
Administrative & Service	15.5	19.0	19.0
Total	166.0	187.0	210.0

20. Comments

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PROPOSAL AND AUTHORIZATION
FOR RESEARCH OR DEVELOPMENT

NYO-149

1. Project Title: Radiation Effects on Biological Systems - Medical Research			2: Date: May 1959
3. Budget Activity No. 6120	4. Budget Item No.	5. Contractor's No.	6. Method and Time of Reporting Progress: Quarterly & Special Reports
7. Contractor: Associated Universities, Inc. Brookhaven National Laboratory		8. Working Location: Upton, New York	9. Contract No. AT-30-2-GEN-16
10. Persons in Charge: L. B. Parr, M.D. (See individual project proposals)		11. Starting Date: Continuing	

SUMMARY

PROPOSAL AND AUTHORIZATION
FOR RESEARCH OR DEVELOPMENT

HYO-149

1. Project Title: Radioactive Elements in Organs and Tissues of Man			2. Date: May 1959
3. Budget Activity No: 6120-1	4. Budget Item No:	5. Contractor's No.	6. Method and Time of Reporting Progress: Quarterly & Special Reports
7. Contractor: Associated Universities, Inc. Brookhaven National Laboratory		8. Working Location: Upton, New York	9. Contract No: AT-30-2-GEN-16
10. Persons in Charge J. S. Robertson, S. H. Cohn, C. J. Shellabarger			11. Starting Date: Continuing

	F. Y. 1959	F. Y. 1960	F. Y. 1961
Cost (in thousands of dollars)	140	145	150
Direct Man-Years			
Scientific	3.2	3.3	3.6
Others	6.9	7.0	7.1
Total	10.1	10.3	10.7

Background, Status and Future Plans:

The human body normally contains radioactivity. The significance of the radiation exposure coming from these radioisotopes is dependent upon the element, its biochemical effects, its cellular location, and the nature of its radioactive emissions. Since evolution has occurred in the presence of small amounts of radiation, the exposure resulting from these so called naturally occurring radioactive isotopes is presumed to be within tolerance limits. It is clear, however, that with the development of atomic energy man's environment in terms of radiation exposure is changing. Further contamination of the environment by a wide spectrum of radioactive elements, some of which will gain access to the interior of the body, will undoubtedly occur. The significance, or indeed the presence of this body contamination remains to be discovered. To determine the amount, and attempts to deduce the significance of radioactive isotopes in the human body from whatever source is the concern of this study.

(a) Whole-Body Counting Facility and Radiochemistry Laboratory
J. S. Robertson, S. H. Cohn, R. L. Cranny

Construction of the whole-body counting facility was completed and the gamma spectrometer was calibrated. The first studies with this facility concerned the determination of the turnover time of I^{131} labeled protein compounds and the uptake of I^{131}. A number of control patients were surveyed in order to provide a base line for the normal levels of internally deposited radionuclides.

A low-level radiochemical laboratory was set up, and a number of radiochemical procedures were standardized. In addition to this facility, a low-level anti-coincidence type beta counter is now under construction by the Instrument Branch.

Studies on skeletal growth and mineral metabolism were performed. It was determined that x-irradiation decreases the rate of accretion of radiostrontium into the non-exchangeable fraction of bone and increases the exchangeable fraction of bone calcium.

A survey of representative groups of persons with the whole-body gamma spectrometer will be undertaken in order to provide data on the level of internally deposited radionuclides in the population. Clinical studies utilizing the gamma spectrometer will be continued, employing gamma emitters such as Sr^{85}, Na^{22}, and hopefully, Ca^{47}. Emphasis will be placed on skeletal mineral metabolism in various disease states of the patient. In addition, a number of collaborative studies will continue, involving the determination of the turnover rates of labeled protein and other radionuclides in various disease states. The low level-radiochemistry

(See continuation sheet)

(a) Whole-Body Counting Facility and Radiochemistry Laboratory (Continued)

service will be continued, primarily for the determination of levels of Sr^{90}. Studies on the growth and metabolism of bone will be continued using animals. The effect of both external radiation and radiation from internally-deposited radionuclides (Sr^{90}) on the rates of accretion and exchange of the skeleton will be pursued. Investigation will be made of the possibility of in vivo Sr^{90} counting by suitable modification of the whole body counting facility to utilize the Bremsstrahlung effect. The object is to develop a technique for external Sr^{90} counting which will be suitable for a population survey similar to that planned for gamma-emitting radionuclides. Prerequisite for this determination is the accurate measurement of the low energy region of the gamma spectra of internally-deposited isotopes. (Successful development of this technique requires the services of a physicist with considerable experience in gamma spectroscopy.) Many of the biological half-times for radionuclides in the whole body and in various tissues should be re-evaluated. Whenever appropriate, this problem will be investigated with the use of the whole-body and gamma spectrometer facilities.

Related research in the Brookhaven National Laboratory program concerns the Marshall Island Survey. As part of the 1959 Marshall Island Medical Survey, measurements were made with the whole-body gamma spectrometer of the body burdens of internally deposited fission products among 235 Marshallese persons inhabiting three islands in the Pacific. One hundred urine samples obtained from the Marshallese people, and samples of plants and animals collected on the Marshall Islands are presently being analyzed for the presence of Sr^{90} and other radionuclides.

(b) Gastric Secretion of Halides J. S. Robertson

Previous and as yet unpublished data obtained in studies of the rates of secretion of Cl, Br, At and I by the gastric mucosa in rats indicated that the differences observed in the apparent secretion rates might be as much due to differences in rates of reabsorption as to actual differences in rates of secretion

Completion of calculations based on the previous data and initiation of a program extending these studies to human beings is contemplated.

(c) The Mathematical Basis for the Interpretation of the Behavior of Tracers
J. S. Robertson

Application of mathematical models in the interpretation of the kinetics of events observed in the bone marrow during tracer studies with tritiated thymidine was attempted, but construction of a completely satisfactory model is yet to be achieved. The complexity of problems of this nature and others which have been encountered introduce higher order equations which exceed the power of analysis in terms of simple models which can be analyzed by desk-type computations. The use of electronic analog computers in the synthesis and analysis of appropriate model systems provides a way to cope with these difficulties. A four-compartment analog computer has been used in some analyses, but this instrument's capacity is extremely limited. An eight-compartment analog computer which will provide greatly increased flexibility is under construction.

It is expected that the analog computer will be completed and its use applied to the back log of existing problems as well as to new ones which arise. Additional equipment, such as a widescreen oscilloscope and a magnetic tape input, is desired to promote maximal usefulness of the analog computer. Programs for refining calculations made with the analog computer by use of digital computers will be developed.

(d) Radiation Induced Carcinogenesis - C. J. Shellabarger, V. P. Bond
E. P. Cronkite, S. W. Lippincott

At least a probable consequence of radiation exposure to man is carcinogenesis. The fundamental question of the presence or absence of a threshold for radiation exposure before harmful effects occur, is of great importance, both practical and theoretical.

It is felt that the study of the dose-response of carcinogenesis and radiation can be studied, at this time and in part, by utilizing total and partial body X-

(See continuation sheet)

(d) Radiation Induced Carcinogenesis (Continued)

and gamma-radiation, recognizing, however, that the response of various species to particle and electromagnetic radiation may differ. The neoplastic response of the rat breast to total and partial body exposure has proven to be unequivocal and reproducible. This system has yielded the following data:

1. Total body radiation induces neoplasia of the breast in intact or gonadectomized female and male rats.

2. The incidence of female rats with one or more neoplasms of the breast following 400 r of gamma radiation reaches a maximum of 90 per cent, while non-exposed females do not exceed 50 per cent.

3. Exposed males have an incidence of at least 50 percent, while non-exposed males have an incidence that does not exceed 10 per cent.

4. Partial body exposure experiments suggest that the breast tissue must be irradiated in order for the neoplastic response to occur.

5. A linear dose response was observed in the intact female rat between the limits of 25 and 400 r of total body radiation.

The following studies have been started (1-4) and scheduled (5-9).

1. The study of hormonal influences on the neoplastic response of the breast to total body irradiation, including thyroid status, pregnancy, and lactation.

2. The effects of increasing doses of partial body irradiation on the induction of neoplasms in the exposed and nonexposed areas.

3. A pathologic study of the endocrine organisms in tumor and non-tumor bearing rats.

4. The effect of fractionating the total body doses on the neoplastic responses of the breast.

5. Possible interactions of known chemical carcinogens and mutagens with radiation on the induction of breast neoplasms.

6. A study of the interaction of X-ray exposure and I^{131} irradiation on thyroid function and carcinogenesis.

7. An investigation of the carcinogenic effect of neutrons on the breast tissue of rats.

8. Attempts to study the neoplastic response of the rat breast to total body doses below 25 r.

9. The only known experiment in which the data suggest that the threshold might not exist for the carcinogenic effects of irradiation is to be repeated and expanded.

PROPOSAL AND AUTHORIZATION
FOR RESEARCH OR DEVELOPMENT

BNL-149

1. Project Title: Radioactive Isotopically Labeled Cells for Predictions of Life Span, Functions and Progeny			2. Date: May 1959
3. Budget Activity No: 6120-2	4. Budget Item No:	5. Contractor's No:	6. Method and Time of Reporting Progress: Quarterly & Special Reports
7. Contractor: Associated Universities, Inc. Brookhaven National Laboratory	8. Working Location: Upton, New York		9. Contract No: AT-30-2-GEN-16
10. Persons in Charge: V. P. Bond, E. P. Cronkite R. M. Drew, J. S. Robertson		11. Starting Date: Continuing	

	F. Y. 1959	F. Y. 1960	F. Y. 1961
Cost (in thousands of dollars)	430	450	465
Direct Man-Years			
Scientific	7.2	7.4	7.8
Others	21.8	22.6	23.5
Total	29.0	30.0	31.3

Background, Status and Future Plans:

Man and all mammals came ultimately from a single cell. During embryonic growth and continuing until maturity, cell differentiation is predominant as well as multiplication. The specific factors affecting cell differentiation are unknown but are believed to be controlled by specific chemical substances. These substances, while not necessarily identical with the genetic material, may interact with it to produce new cell types, thereafter consistently reproducing. It is of the greatest importance to learn how this genetic material is transmitted from one cell to another and how it may be altered so that in the one instance a new species arises whereas in the other a neoplasm arises. Radioisotopic labeling provides a possible means but it must be firmly established that the radioisotopic label itself does not affect the process observed. Varieties of labels providing quite different energy inputs may give insight into the answers to both questions.

(a) In Vivo Studies with H^3 Thymidine, H^3 Cytidine, and $S^{35}O_4$
E. Cronkite, V. Bond, S. Killmann, P. Reizenstein, E. Usenik, J. Bateman, L. Feinendegen, N. Ordartchenko, and A. Tsuya.

Labeling cells with H^3 thymidine at the time of synthesis of DNA prior to mitosis thus enables one to study migration, pathways, cell transformation, proliferation rates and capabilities, life span, and function of these cells in human beings and animals. Particular attention has been given to normal hematopoiesis and selected leukemias and lymphomatous diseases of man and dogs.

Labeling RNA with H^3 cytidine enables one to follow the relationship of RNA turnover to cell proliferation, function, and transformation particularly in hematopoiesis and during induction of antibody formation. The following findings are noted:

1. Labeling of normal hematopoietic cells in mice, guinea pigs, rats, dogs, and patients was shown to be without demonstrable effect immediately.

2. Upper limits have been established for average stay of the granulocyte in peripheral blood of man.

3. The turnover pattern of hemopoietic cells of human marrow and in peripheral blood of man has been established in part. Small lymphocytes are long lived, perhaps more than 100 days. Medium and large lymphocytes turnover rapidly. The generation time for the last normoblast multiplication is about 22 hours. The generation time of granulocytes is in the vicinity of 18-30 hours. Mitotic index

(See continuation sheet)

(a) In Vivo Studies with H^3 Thymidine, H^3 Cytidine, and $S^{35}O_4$ (Continued)

of normal human marrow between 8 a.m. and 12 noon is 8.97/1000. There is a diurnal variation in mitotic index of human marrow.

4. Under normal conditions a small per cent of circulating cells are synthesizing DNA in all mammals including man so far studied.

5. There is a continual outpouring of immature DNA synthesizing cells from lymphatic vessels into the blood stream. Their fate and function are obscure.

6. No leukemia or blood dyscrasia studied so far has a greater labeling index than normal hematopoietic tissue hence the generation time is probably longer in these diseases. In the case of multiple myeloma the low mitotic index of approximately 1:5000 cells and 1 per cent labeling with thymidine suggests a doubling time of perhaps as long as 300 days. Similar observations have been made in chronic lymphocytic and myelocytic leukemia. In the acute terminal blast crisis

(c) The Temporal Relationships of Deoxyribonucleic Acid Synthesis to Mitosis and the Generative Cycle in Human Cancer Cell (HeLa) Cultures
R. M. Drew and R. B. Painter

The investigation of time relationship of the several components of the generative cycle, utilizing tissue culture of HeLa cells with H^3Th, yield data on the activity and proliferation of cell populations and serve as a model to study radiation effects on cell cycles.

Information concerning the variability in cell DNA synthetic time within different populations of mammalian cells has been obtained. The DNA synthetic time, the resting phase between DNA synthesis and mitosis and the post-mitotic resting phase for HeLa and the HeLa S3 clone cells have been determined. In comparing the aspects of DNA physiology of S3 and HeLa cell cultures, the latter has shown, repeatedly, the persistence of a very small percentage of cells with extremely long DNA synthetic time.

These studies will be continued on normal and malignant cells, and the information obtained will serve as a model for experiments testing the effects of external irradiation on DNA synthesis.

(d) Dynamics of Incorporation of Nucleic Acid Precursors into Cellular Components
V. P. Bond, L. Feinendegen, R. B. Painter, W. Shreeve.

The concern of this study is to measure, by autoradiographic and biochemical techniques, the time rate of incorporation of tritium-labeled nucleic acid precursors into subcellular structures of cells because these data are valuable as basic studies on cell activity and proliferation, and as models for studying irradiation effects on the cell cycle.

Standard biochemical procedures have not allowed clear definition of the precise site and time of synthesis of DNA, RNA, and protein within the cell. High resolution autoradiography with tritium labeled precursors, and cell fractionation and other biochemical techniques, make it possible to localize accurately the sites of synthesis with the cell.

HeLa and Osgood tissue culture cells have been used to date, and the time course of distribution of label after administration of tritium labeled cytidine has been characterized by autoradiographic and biochemical techniques. Preliminary work on other precursors (labeled ribose, deoxyribose, acetaldehyde and thymidine), and on short-term cultures of hematopoietic cells has been done.

Radioactivity from labeled cytidine has been shown to go first to the nucleus, then to the nucleoli, and then to the cytoplasm and out to the medium. The above work will be continued and extended on cytidine and other precursors, in an effort to characterize the course of RNA, DNA, and protein synthesis in normal and malignant cells.

(e) DNA Synthesis and Cell Turnover Activity in the Mouse Embryo
V. P. Bond

Attempts to establish the cell turnover time of embryological tissues is being studied utilizing tritiated thymidine as an estimate of DNA synthesis rate.

No incorporation of H^3 thymidine was noted prior to the establishment of embryonic circulation following which time varying degrees of incorporation in the maternal and fetal structures 24 hours after H^3-thymidine injection were noted.

The investigations will be extended to study uptake of H^3-thymidine in embryonal tissues in fetuses of different degrees of maturity as a function of hours following H^3Th administration to the mother. This will allow an estimation of the turnover rates for individual tissues.

(f) Intranuclear Irradiation with Tritium
J. S. Robertson

Because of its extremely short range in tissues, the tritium beta particle can be used to achieve selective irradiation of cell nuclei. Calculations were made extending previous calculations of the dose rates about a point source of tritium to explain variations in resolution and image spread seen in autoradiographs of tissue sections labeled with tritiated thymidine. Extensive calculations

(See continuation sheet)

(f) Intranuclear Irradiation with Tritium (Continued)

will be made directed toward comparing the ionization distribution in cell nuclei irradiated with tritium with that of cell nuclei irradiated with X- and gamma rays is contemplated as part of the investigation of the RBE of irradiation with the tritium beta particle.

(g) Studies on the Cytochemical Mechanisms of Bone Growth and Repair During Aging, and Skeletal Pathology Including Tumor and Cancer Formation

E. P. Cronkite

These studies are directed at obtaining fundamental information on the biochemistry of cells taking part in vital activities leading to the growth and repair of bone and at ascertaining the deviation of the normal biochemistry of these cells as a result of skeletal pathology. In attempts to alleviate many of the pathological conditions which are associated with the skeletal system, there is the need of obtaining fundamental cytochemical information on the role of bone cells and their activities in the normal animal, and which in turn, this information can be used as a base line for comparison and a better study of skeletal pathology.

The utilization of histochemical and autoradiographic techniques on the following problems is planned.

1. To study the contribution of the various cells and tissues to fracture healing using tritium labeled thymidine on rats, from birth to old age.

2. The mode of osteoclast formation, disappearance and histocytochemical role using tritium labeled thymidine.

3. The localization, distribution and function of ATP-ase in bone formation during aging.

4. Protein formation in osteoblasts using H-labeled amino acids.

5. Development of combined radioisotope methods with histochemical techniques to allow more precise quantitative analyses of the stained cell and time components.

(h) Studies of the Metabolic Pathways of DNA and Its Precursors in Radiation Injury and During Radio- and Chemotherapy

E. P. Cronkite, V. P. Bond, S. Killmann, and P. Reizenstein

Degradation and pathways of labeled DNA in normal and malignant cells is vital to evaluation of radiation in injury and certain types of therapy just as the metabolic pathways of DNA precursors vital to understanding normal and neoplastic cell growth. More data are needed on the availability, time and fate of injected H^3 thymidine in patients and animals.

The following findings are to be noted:

1. Plasma clearance of H^3 thymidine and degradation pathways were established in normals and patients with leukemias.

2. Appearance of labeled beta aminoisobutyric acid (BAIBA) in urine of patients was established.

3. Excretion of BAIBA in radiation injury of man and animals was shown. The quantitative excretion of BAIBA in the Oak Ridge casualties appeared dose dependent.

4. Excretion patterns of BAIBA in leukemics during and between therapeutic regimes have been established.

5. The excretion of BAIBA has been correlated with low mitotic index (low cell turnover) and with a possible shunt of thymine to BAIBA suggesting a constant endogenous level of thymidine production and a spill over into the thymidine-BAIBA pathway when DNA synthetic pathway is blocked or eradicated.

These studies are to be continued.

PROPOSAL AND AUTHORIZATION
FOR RESEARCH OR DEVELOPMENT

NYO-149

1. Project Title: Hematology of Radiation			2. Date: May 1959
3. Budget Activity No: 6120-3	4. Budget Item No:	5. Contractor's No:	6. Method and Time of Reporting Progress: Quarterly & Special Reports
7. Contractor: Associated Universities, Inc. Brookhaven National Laboratory	8. Working Location: Upton, New York		9. Contract No: AT-30-2-GEN-16
10. Persons in Charge: E. P. Cronkite		11. Starting Date: Continuing	

	F. Y. 1959	F. Y. 1960	F. Y. 1961
Cost (in thousands of dollars)	20	21	22
Direct Man-Years			
Scientific	0.2	0.2	0.2
Others	1.3	1.4	1.5
Total	1.5	1.6	1.7

Background, Status and Future Plans:

One of the major findings following radiation exposure is the profound depression of formed elements of the blood. Understanding of the factors that control the circulating levels of the formed elements of the blood must go hand-in-hand with, or proceed, the understanding of the effect of radiation on these processes in health and diseased states.

High levels of a physiologically active substance, serotonin, are found in blood platelets and parts of the gastrointestinal tract. Serotonin levels of tissues and platelets will be measured colorimetrically from tissues of normal and irradiated animals and platelet preparations to assess further the role of this substance in radiation injury and the effectiveness of platelet preparations in controlling radiation hemorrhage.

Urine levels of 5-hydroxyindoleacetic acid, the excreted metabolite of serotonin, will be measured in normals, leukemics, and other blood dyscrasias.

Hemostasis in irradiation injury by transfusion of fresh platelets is an accomplished fact. The mechanism of action of the platelets remains obscure. $S^{35}O_4$ labeling of platelets, coupled with autoradiographic techniques suggests that a definite platelet-endothelial interaction may occur.

Attempts to label platelet RNA with H^3 cytodine have been begun in an effort to study this problem.

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PROPOSAL AND AUTHORIZATION
FOR RESEARCH OR DEVELOPMENT

NYO-149

1. Project Title: Radiation Effects on Immunity and Allergy			2. Date: May 1959
3. Budget Activity No: 6120-4	4. Budget Item No:	5. Contractor's No:	6. Method and Time of Reporting Progress: Quarterly & Special Reports
7. Contractor: Associated Universities, Inc. Brookhaven National Laboratory		8. Working Location: Upton, New York	9. Contract No: AT-30-2-GEN-16
10. Persons in Charge: R. D. Stoner, G. Terres and W. Wolins		11. Starting Date: Continuing	

	F. Y. 1959	F. Y. 1960	F. Y. 1961
Cost (in thousands of dollars)	160	163	168
Direct Man-Years			
Scientific	2.5	2.5	2.5
Others	8.9	9.0	9.3
Total	11.4	11.5	11.8

Background, Status and Future Plans:

Radiation depresses antibody production while at the same time anaphylactic allergy is enhanced. During the recovery period of antibody production anaphylactic allergy may decrease to normal levels. Presumably a single system of protein synthesis and cell reactivity is responsible for this paradox. The concern of this study is to investigate antibody production, particularly in regard to the effects of radiation.

(a) Mechanism of Immune Reaction
R. D. Stoner, S. Killmann, V. P. Bond, E. P. Cronkite

These studies have a dual function: (a) to shed light on the fundamental problems of immunity and anaphylaxis and (b) to provide information on the effects of radiation on these processes particularly in regard to the depressant effects of radiation on antibody production and the enhancing effects of radiation on anaphylaxis. The following studies have been carried out:

1. Comparative Radiosensitivity of the Primary and Secondary Antibody Response. Preliminary findings indicate that the primary tetanus antitoxin response in mice is more sensitive to radiation than is the secondary antitoxin response. Graded doses of radiation are being used to compare the radiosensitivity of the antibody-forming mechanism in normal mice and in mice that have experienced a primary antibody response.

2. Depressant Effect of Acute, Divided Acute and Chronic Exposure to Radiation on Antibody Formation. Equal total doses of radiation are given to animals in a single acute exposure, divided acute exposures and chronic exposure. The secondary tetanus anti-toxin response is used to measure the depressant effect of radiation under these conditions.

3. Radiation and Time of Antigenic Stimulus. The depressant effect of radiation on the secondary antibody response is closely associated with the time of injection of the secondary antigenic stimulus. A detailed study is being made of the secondary antigenic stimulus in relation to acute, divided acute and chronic exposure to radiation.

4. Enhancing Effect of Radiation on Anaphylaxis. Although antibody production is suppressed following exposure to radiation an increasing susceptibility to fatal anaphylaxis appears after radiation. A continuing study is being made in an attempt to explain this phenomenon.

(See continuation sheet)

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(a) Mechanism of Immune Reaction (Continued)

5. Radiosensitivity of Tissue. Work is continuing in study of the sensitivity of intraocular transplants of antibody forming tissues to beta and gamma radiation. The secondary antibody response from the transplanted tissue is used to measure the radio-sensitivity of the various tissues.

6. Effect of Internal Radiation on Antibody Formation. Continuing experiments are being carried on to study the effects of yttrium-90 on the secondary antibody response in mice. These studies will be extended to evaluate the effects of strontium-90 on immune mechanisms.

7. Cell Proliferation in Lymphoid Tissue During the Antibody Response. Tritium labeled thymidine is being used in study of the cells that may arise or proliferate during the secondary antibody response. Preliminary experiments are being carried on at the present time.

All studies indicated above will be continued with priority decreasing in order of increasing number.

(b) Acquired Immune Tolerance to Crystalline Bovine Serum Albumin in Mice
G. Terres

This study is based on the finding that animals neonatally exposed to an antigen become specifically immunologically tolerant or unresponsive to the antigen. Data accumulated will provide insight into the normal immune response, into antibody synthesis, and into the mechanism initiating auto-immunity and particularly to the fundamental mechanism by which native proteins are recognized and thus fail to elicit an antibody response.

Mice were exposed to the antigen during the first two weeks of life. At six weeks of age the mice were re-exposed to the antigen and subsequently challenged. Mice similarly treated were bled. To the serum was added I^{131}BSA and the gamma globulin precipitated with ammonium sulfate. The per cent radioactivity precipitated (antigen-antibody complexes) was determined. The incidence of anaphylactic sensitivity and the per cent of I^{131} precipitated with ammonium sulfate was significantly lower in mice exposed to the antigen during the first two weeks of life. These observations will be extended by testing the serum of older mice which had been postnatally exposed to the antigen. Also, the development of sensitivity with time will be more accurately defined in mice exposed to the antigen at birth. Various tissues from immunologically tolerant mice will be isolated and tested for BSA using I^{131} or H^3 labeled antibodies and radioautography.

(c) Immune Degradation
G. Terres and W. Wolins

The concern of this study is to characterize the kinetics of the antigen-antibody reaction in vivo in order that a method may be developed so that in diseases where an auto-immune mechanism has been implicated can be investigated.

Antigen was labeled with I131 and mice were passively sensitized with rabbit anti-serum. The amount of antigen degraded at an accelerated rate was a function of the amount of antigen and antibody used. With one antiserum, the initial rapid degradation phase was followed by one in which the antigen was degraded at a rate slower than in normal mice. Preliminary studies were also started with horse and mouse antiserum.

The reverse experiments are to be done in which the antibody is labeled and its degradation followed as a function of the amount of antigen used. Also an experiment is planned in which the antigen is labeled with I^{131} and the antibody with I^{131}. Thus the degradation of both can be followed simultaneously. Studies in which horse and mouse antisera are used to be completed.

(d) Antibody Absorption on Guinea Pig Ileum
G. Terres

The nature of the binding between antibody and tissue is being studied to learn more about the quantitative relationship between the amount of antibody absorbed and the degree of sensitization.

Here, antibody is labeled with I^{131}. Normal guinea pig ileum is soaked in the protein and the amount of radioactivity absorbed is measured as well as the

(See continuation sheet)

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(d) Antibody Absorption on Guinea Pig Ileum (Continued)

degree of sensitization thus attained. The sensitization is measured by the Schultz-Dale reaction.

It has been found that the amount of antibody is proportional to the concentration of protein used and conforms to a Langmuirilan isotherm. The sensitivity as determined by the Schultz-Dale reaction is proportional to the amount of protein (aintibody) absorbed.

It is intended to measure nore accurately the protein absorbed by using autoradiography. Thus the amount of protein absorbed per type of cell can be determined relative to the degree of sensitivity imparted.

(e) An Attempt to Show the Existence of Normal Auto-Antibodies
W. Wolins, G. Terres and C. J. Shellabarger

Several disease states in man have been shown to be consequent to autoimmune mechanisms. Other diseased states for which auto-antibodies have not as yet been demonstrated are believed to be auto-immune in origin. It seems conceivable that auto-antibodies may be normally formed rather than a state of immune un-responsiveness to ones own tissues, and that these antibodies, by virtue of quantitative or qualitative differences, induce no pathology.

One can modify the concept of immune tolerance to include degrees of autoimmune reactivity and postulate that pathology dependent on an altered immune state. This reactivity to auto-antigens may be present from the onset of the antibody producing responses but the presence of auto-antibodies is inapparent because they unite rapidly with their corresponding antigen. An excess or an alteration in the nature of the antigen can then induce disease.

(1) Athyreotic female rats are bred, pregnancy maintained with thyroxin. (2) Newborn rats are made athyreotic by I^{131} in doses adequate to produce thyroid ablation, (3) Growth and development of these rats are maintained by exogenous thyroxin, (4) At maturity rats are to be given whole body radiation adequate to suppress the immune response for several weeks. (5) Implants of lymph nodes from normal rats are to be introduced into the athyreotic irradiated group. (6) After several weeks these rats are to be bled and the presence of antibodies directed against thyroglobulin or thyroid epithelium are to be determined by the sensitive tanned red cell technique, or by complement fixation.

PROPOSAL AND AUTHORIZATION
FOR RESEARCH OR DEVELOPMENT

NYO-149

1. Project Title: Radioisotopic Tracing of Total and Intermediate Carbohydrate Metabolism			2. Date: May 1959
3. Budget Activity No: 6120-5	4. Budget Item No:	5. Contractor's No:	6. Method and Time of Reporting Progress: Quarterly & Special Reports
7. Contractor: Associated Universities, Inc. Brookhaven National Laboratory		8. Working Location: Upton, New York	9. Contract No: AT-30-2-GEN-16
10. Persons in Charge: W. W. Shreeve, I. Schwartz, P. Le Fevre		11. Starting Date: Continuing	

	F. Y. 1959	F. Y. 1960	F. Y. 1961
Cost (in thousands of dollars)	220	230	240
Direct Man-Years			
Scientific	3.9	4.0	4.0
Others	10.9	11.0	11.3
Total	14.8	15.0	15.3

Background, Status and Future Plans:

Carbohydrate metabolism may be modified in various disease states, and by some hormones and drugs. The utilization of C^{14} or tritium labeled carbohydrates aids in the study of the rate and metabolic pathways of the synthesis and degradation of carbohydrates. Since carbohydrate metabolism and fat metabolism are interrelated, a new program has been started to investigate the hormonal regulation of fat metabolism in the radiation syndrome. Further studies on the kinetics and molecular specificity of sugar transfer through cell membranes utilizing labeled materials aid the understanding of carbohydrate metabolism.

(a) Studies on Diabetic and Non-diabetic Human Subjects
W. Shreeve and R. DeMeutter

Experimental studies were conducted with these patients using 1-C^{14}-acetate, 2-C^{14}-acetate, or 2-C^{14}-pyruvate. Total isotope used was about 500 microcuries. With the patients in various states of diabetes, fasting, or corticosteriod excess, the rate and pathways of glucose formation were estimated by C^{14} analyses of blood glucose. In addition the rate of the labeled compounds was studied more widely by analysis of C^{14} in breath CO_2 and blood bicarbonate, plasma triglycerides and cholesterol, plasma alpha-keto acids, and urinary beta-keto acids. Positive findings included an indication of an unusual pathway of glucose formation form pyruvate, depressed CO_2 formation from acetate as an effect of corticosteroid administration, and decreased cholesterol and triglyceride formation from acetate in the elderly, stable type of diabetic. Other studies with diabetics included C^{14} analyses of blood glucose for interpretation of rate and pathways of hepatic glucose formation. The effects of insulin, sulfonylureas, and glucose load on these processes are being studied in each diabetic patient. In order to understand more fully the fate of lactate and pyruvate in relation to glucose formation breath CO_2 and blood lactic acid have also been analyzed for C^{14} content. Findings so far include a quite definite decrease in formation of C^{14}-labeled blood glucose as an effect of insulin, and further indications that metabolic reactions forming glucose from lactate or pyruvate are more diverse than supposed.

It is expected that the program with human diabetics will continue at about the same pace and in the same direction as the previous year. Findings on both rate and pathways of glucose formation under various hormonal, nutritional, and drug influences need to be confirmed and enlarged. The program has been generally hampered in its estimate of the rate of oxidation of labeled intermediates by depending on intermittent analysis of breath samples by a rather crude method. A continuous $C^{14}O_2$ recording instrument for breath analysis will be acquired. Immediate and continuous records of respiratory C^{14} activity and cumulative C^{14} excretion will ultimately be available. Later addition of other equipment will provide $C^{14}/C^{12}O_2$ ratios and thus specific $C^{14}O_2$ activities.

(See continuation sheet)

(b) Animal Studies of Sugar Metabolism
W. Shreeve and R. De Meutter

In this year also there was completed a study (begun in the previous two years) of ribose and deoxyribose formation from C^{14}-labeled glycine in regenerating rat liver. Results indicated that at least part of deoxyribose in DNA had an origin different from that of ribose in RNA. There has been some work this year on the metabolism of pentoses. Along with the nucleosides, T-thymidine and T-cytidine, some labeled sugars (T-deoxyribose and C^{14}-ribose) have been presented to tissue culture cells followed by radioautography and chemical analyses of nucleic acid fractions. With regard to sugars the essential finding has been that deoxyribose was a poor precursor of DNA, while ribose was converted extensively to both RNA and DNA

Research collaborators from Columbia University spent two months of this year in the Biochemistry Division getting started on a study with C^{14} of the pathways of carbohydrate utilization in the electric eel in order to relate the unique form of energy produced to the nature of the metabolism producing it. Present findings indicate an extensive use of the glucose oxidative pathway in this species.

There is some possibility that the study of pentose formation will be renewed using rat liver and tissue culture cells. A survey of various special and common labeled precursors for relative formation of deoxyribose of DNA and ribose of RNA is contemplated.

(c) Radiation Physiology of Blood Lipids and Adipose Tissue
I. Schwartz

Effects of radiation on the storage and transport forms of body fat represent virtually unexplored facets of radiation physiology. Because fat is one of man's primary metabolic fuels and a major constituent of his body, an investigation of the changes in lipid metabolism and lipid structure resulting from various forms of radiation offers promise of new insight into the nature of radiation toxicity.

Standard methods of lipid analysis have been set up. A preparation of aluminum-bound C^{14} palmitic acid has been prepared to serve as a tracer for the pool of non-esterified fatty acids.

Following total body irradiation in dogs and laboratory mammals, measurements of radiation effects on the composition of selected blood lipids will be made.

(d) Radioisotopic Analysis of Lipid Metabolism and Transport in the Blood
I. Schwartz

The availability of isotopic fatty acids has greatly facilitated the study of the mobilization, transport and utilization of body fat. Only recently it has become clear that the non-esterified fatty acid (NEFA) fraction of plasma is a highly important metabolic fuel which alone can supply as much as 50% of the total energy requirement of fasting human beings. (This fact was established directly by determination of $C^{14}O_2$ in expired air after administration of C^{14}-labeled NEFA to man and dogs). Thus the NEFA fraction of plasma is capable of delivering energy to working cells at a rate equal to and possibly exceeding the caloric flux available from glucose. What is even more interesting is the fact that delivery of NEFA from storage is regulated to supply caloric deficits arising when the total energy requirement exceeds the energy made available from carbohydrate metabolism. This regulation is rapid, sensitive, and involves versatile attributes of adipose tissue that were unsuspected until very recently. It is of considerable importance to make use of isotopic fatty acids to characterize the nature of this regulation not only because of its central position in caloric homeostatis, but also because of the high probability that an unregulated flow of NEFA from storage is the cardinal defect which causes diabetic ketosis. For this reason, studies of the regulation of plasma NEFA levels have been combined.

The nature of the *in vivo* elevation of plasma NEFA levels by epinephrine and thyroid hormones in human subjects and animals will be investigated.

(See continuation sheet)

(e) Transfer of Metabolites Through Cell Membranes

P. Le Fevre, G. McGinniss

Kinetics of Sugar Transfer - New theoretical calculations derived from alternative current interpretations of kinetic data on net sugar transfers in the red cell have led to a previously unrealized application of isotopic tracer methods, providing decisive means for discriminating between the proposed hypotheses. Previous kinetic studies in this area have all dealt with net transfers in response to gross concentration gradients. The saturation kinetics found with D-glucose and related monosaccharides has been attributed either to operation of a special sugar carrier system, or to non-specific depression of cell permeability by surface alterations at high sugar concentrations. The latter hypothesis predicts a tracer glucose exchange rate which is only a small fraction of that predicted by carrier theory. Therefore, C^{14}-glucose equilibration in both directions was studied in thick suspensions of human erythrocytes. The speed of tracer equilibration in comparison with the speed of net transfer was 50-100 times greater than would be found in a diffusion process; these high fluxes are consistent with the carrier theory--provided that a glucose-carrier affinity appreciably higher than that previously estimated be assumed.

Molecular Specifity in Sugar Transfer Systems: The likely extension of the confromational type of biological stereo-specifity discovered in the human red cell to other monosaccharide transfer systems was investigated, with special attention to the mouse Krebs ascites tumor cell. This cell is not adaptable to the Orskov densitometric procedure used for the erythrocyte studies, and appropriate chemical methods have been developed for improved quantification of the partial results reported from Cori's laboratory in 1956. Estimation of the specific carrier constants in this cell, which will permit consideration of the correlation with molecular conformational stabilities, is beginning.

The related problem of the reversible combination of these systems, in erythrocytes and ascites tumor cells, with phleretin, stilbesterol, hexestrol, and other such diphenolic competitive inhibitors, has been extensively studied. Various correlations have been drawn in respect to minor rearrangements in these diphenolic molecules and alterations in the composition of the medium, as factors in the degree to which the agents become fixed to the cells. These considerations may assist in identification of the reactive groups in the presumed carrier, a matter which has thus far defied attack in several laboratories.

Projected Studies Include:

1. Sterospecifity in the mouse Krebs ascites tumor cell (and related cell types) sugar-transfer systems, as initiated above.

2. Extension of above studies on specific inactivation of carrier's reactive groups, and the associated fixation of inhibitors; attempt to delineate critical molecular assembly involved.

3. Systematic densitometric study (with esmometric quantitative control) of absolute rate constants from transfer of sugars with wide range of carrier affinity; since preliminary estimates suggest rate constant may be invariant, this would constitute strong new evidence for current working model for carrier system.

4. C^{14}-ribose (or other low-affinity sugar) tracer equilibration studies, for contrast with above glucose tracer studies; if consistent with theoretical prediction, certainty of interpretation of glucose tracer behavior would be greatly strengthened.

PROPOSAL AND AUTHORIZATION
FOR RESEARCH OR DEVELOPMENT

HYO-149

1. Project Title: Radioisotopes for Study of Protein and Nitrogen Metabolism			2. Date: May 1959
3. Budget Activity No. 6120-6	4. Budget Item No.	5. Contractor's No.	6. Method and Time of Reporting Progress: Quarterly & Special Reports
7. Contractor: Associated Universities, Inc. Brookhaven National Laboratory		8. Working Location: Upton, New York	9. Contract No: AT-30-2-GEN-16
10. Persons in Charge: D. D. Van Slyke, W. L. Hughes, M. Maxfield		11. Starting Date: Continuing	

	F. Y. 1959	F. Y. 1960	F. Y. 1961
Cost (in thousands of dollars)	170	174	180
Direct Man-Years			
Scientific	5.5	5.6	5.9
Others	6.6	6.7	6.8
Total	12.1	12.3	12.7

Background, Status and Future Plans:

The use of labeled proteins for an evaluation of the nutritive state of intact organisms will continue to be explored. C^{14} and tritium labeled compounds give information on a nutritive condition and food utilization, and further demarcate broad pathways into which come and go many other elements. For example, tritium emits very weak beta particles with a range less than cellular dimensions. Thus, this isotope is useful for locating labeled compounds as well as possibly localizing radiation effects within a given cell or part of a given cell.

(a) Mechanisms of DNA Synthesis
W. L. Hughes

The mechanism of re-duplication and subsequent division of the genetic material of cells can be studied utilizing tritium labelled thymidine as a tracer for DNA, the chief component of the genetic material.

The primary cause of radiation injury within the cell is presumably the effect on DNA. The previous studies on the mechanism of DNA duplication, utilizing tritium labeled compounds is to be continued and extended utilizing deuterium compounds as they are synthesized and become available for use..

(b) The Preparation and Use of Tritiated Compounds for Bio-medical Studies
W. L. Hughes, H. A. Johnson, R. B. Painter, L. Feinendegen

Tritiated thymidine has become a valuable aid in the study of intranuclear radiation damage. A wide variety of experimental material has been used to assess the radiation damage that may occur as the result of placing radioactivity within the nucleus. Specific attempts to study this problem include: 1) Utilization of tissue culture techniques to assess the radiation effects on cellular division, 2) Attempts to relate radiation dosage to chromosome breakage, utilizing plant material in cooperation with collaborators, 3) In intact animals, utilizing radioautographic techniques to locate the sites of incorporation of tritiated thymidine and estimation of the dosage delivered, and then searching for radiation effects. The radiation effects appear to be small, temporary sterilization and some thymic weight loss are the only effects observed so far. Data of this nature are, of course, quite important in assessing the potential hazards from contamination with this material. 4) Replacement of thymidine with iodo (I^{131}) deoxyuridine in DNA synthesis makes possible the tracing of DNA synthesis with a gamma emitting isotope, which can be counted from outside the body in intact organisms. Thus this compound will be explored as to its usefulness in locating dividing cells, in tumors, for example, or in assessing radiation damage where it depresses cell division, or where chemotherapeutic agents might be expected to depress cell division. 5) Radiation induced cataract formation presumably results from effects upon the epithelium of the lens. Studies on the renewal of the lens epithelium

(See continuation sheet)

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(b) The Preparation and Use of Tritiated Compounds for the Bio-medical Studies (Continued)

have been initiated utilizing tritiated thymidine. 6) Other tritiated components such as cytidine and arginine have been produced. Studies of these components in tissue culture have been initiated. In addition, serum albumin labeled with tritium and this material will be used to study sites of antigen antibody reaction by use of autoradiography.

(C) Plasma Protein Metabolism
W. L. Hughes

Serum albumin has been labeled with tritium, and serum lipoproteins have been labeled with radioiodine. The turnover of these compounds will be studied by counting techniques and the sites of catabolism will be localized by radioautography, as part of the general program on the replacement of serum proteins.

(d) Metabolism of C^{14}-labeled amino acids by Trichinella Spiralis Larvae
L. V. Hankes, R. D. Stoner

Investigation of the problems of trichinosis as studied with radioactive compounds yields information on host-parasite relationships. Specifically, the effects of an active trichinella infection on host metabolism is being investigated. In vitro turnover studies of labeled proteins and carbohydrates are being studied in Trichinella larvae, utilizing larvae cultures in chemically defined medial containing C^{14}-labeled amino acids. In vivo studies include the feeding of C^{14}-labeled amino acid diets to Trichinella infested mice. Tissue and blood components were analyzed for C^{14} to assess the effects of parasitic infection on host metabolism. These studies are to be continued.

(e) Studies on the Glycoprotein Orosomucoid
E. A. Popenoe and M. Maxfield

Among the plasma proteins, the low molecular weight, acidic glycoproteins are more or less unique in that no specific functions have yet been attributed to them. It is unlikely, however, that they are mere chance constituents. The concentration in the plasma of the best characterized of these glycoproteins, orosomucoid, is markedly elevated in a number of apparently unrelated conditions including pregnancy, rheumatoid arthritis and various neoplastic diseases. In the latter diseases, in fact, the plasma orosomucoid level can be used as an indication of a patient's response to therapy. Where treatment has been successful the level drops. Because orosomucoid is inherently an interesting substance from the protein chemist's point of view and because it is becoming increasingly important clinically, a study of its structure has been undertaken and is continuing. This glycoprotein is about forty per cent carbohydrate and sixty per cent protein. The carbohydrate portion contains about 17 residues of sialic acid per protein molecule and each of these residues has been shown to be "terminal", that is, linked to the rest of the molecule through a single glycosidic bond. An enzyme produced by the microorganism Clostridium welchii can remove all of these sialic acid residues without any other detectable effect on the glycoprotein molecule. Each sialic acid residue has been found to be linked to a galactose residue, probably to the number 3 carbon atom. Experiments seem to indicate that the carbohydrate portion of the glycoprotein is a single polysaccharide unit, and since it has at least 17 "end groups" this polysaccharide must have a highly branched structure.

The "turnover" studies will be continued and extended to as many patients as seems advisable and practical. The probing into the structure of Orosomucoid will continue. It would appear now that this study will involve (1) an enzymatic approach using glycosidases from various sources to effect specific cleavages and (2) attempts to destroy the polypeptide part of the molecule by various chemical treatments in such a way that only those amino acids linked directly to the polysaccharide remain. The purpose of this latter approach is to discover the carbohydrate-to-protein link which is as yet unknown.

(f) Radioisotopes for Study of Protein and Nitrogen Studies of Collagen
D. D. Van Slyke, O. P. Foss, E. Popenoe, L. Commerford

Collagen is the structural protein that binds tissue components together. It comprises a large part (in the rat forty per cent) of mammalian body protein. It is involved in the changes of the "collagen diseases" and of aging. The conditions

(See continuation sheet)

(f) Radioisotopes for Study of Protein and Nitrogen Studies of Collagen (Con'd)

of collagen formation and turnover are being studied in rats. A tool for such studies is provided by the amino acid, hydroxylysine, which is unique in that it occurs only in collagen. As shown by experiments with C^{14}- and H^3-labeled lysine and hydroxylysine in this Laboratory, the only source of the hydroxylysine in collagen is lysine, which is absorbed from digested proteins and hydroxylated when, or shortly after, it is incorporated into collagen. Administered hydroxylysine itself is not incorporated. Studies are in progress concerning the processes of incorporation and hydroxylation of lysine in collagen, the turnover rate of collagen at different ages and in different tissues, and the function of hydroxylysine in the collagen molecule. Only part of the hydroxyl groups of hydroxylysine in collagen appears to be free, twenty-five per cent or more in collagen from different tissues being masked by coordinate or other type of bonding, which may be essential to the structure of the collagen molecule. It is intended that the studies on the above factors under normal conditions will be later extended to cover the effects of pathological conditions and radiation injury.

(g) Relationships among Urinary Mucoproteins
M. Maxfield

The molecular weight, size and shape of the DiFerrante urinary mucoprotein which had been obtained from light scattering studies have been confirmed by ultracentrifuge and viscosity studies. The urinary mucoprotein of Tamm and Horsfall has been studied in water solution with ultracentrifuge and viscosity, confirming the previously determined molecular weight of 25×10^6. This molecular weight is in agreement with the light scattering studies in water solution and with electron microscope pictures. It has been shown that this form of the mucoprotein of Tamm is in reversible equilibrium with the 7×10^6 molecular weight form reported by Tamm. In distilled water the 25×10^6 form exists alone. Upon dilution with water, the mucoprotein dissociates into the 7×10^6 form. In the presence of salt, the 7×10^6 form is in equilibrium with tactoids. Upon standing, the tactoids further aggregate into a gel. The mucoprotein of Tamm may be dissociated irreversibly by cetyl into a homogeneous component of 1.6×10^6 molecular weight. This is one-half of the molecular weight of the DiFerrante mucoprotein. Of the various forms described, the Tamm and the DiFerrante mucoproteins are active virus inhibitors.

Continued investigation of the various forms of urinary mucoprotein is proposed. An effort will be made to determine the antiviral activity of each of the forms of mucoproteins and the relationship of the mucoprotein to the formation of kidney stones will be investigated further.

(h) Effect of Radiation on Mucoprotein Physiology
M. Maxfield

Normally, the amount of mucoprotein excreted in the urine of normal dogs is similar to that of man, as is the specific viscosity. (The specific viscosity is a measure of axial ratio of the mucoprotein molecule). Daily samples of urine from one dog which received 550 r whole body radiation were studied. In this single example the amount of urinary mucoprotein excreted and the specific viscosity were both reduced during the period of the acute radiation syndrome. This indicates that in this dog an abnormal type of urinary mucoprotein was excreted due to the irradiation. This line of study will be continued.

PROPOSAL AND AUTHORIZATION
FOR RESEARCH OR DEVELOPMENT

NYO-149

1. Project Title: Clinical Management of Radiation Injury			2. Date: May 1959
3. Budget Activity No: 6120-7	4. Budget Item No:	5. Contractor's No:	6. Method and Time of Reporting Progress: Quarterly & Special Reports
7. Contractor: Associated Universities, Inc. Brookhaven National Laboratory	8. Working Location: Upton, New York		9. Contract No: AT-30-2-GEN-16
10. Persons in Charge: E. P. Cronkite, V. P. Bond	11. Starting Date: Continuing		

	F. Y. 1959	F. Y. 1960	F. Y. 1961
Cost (in thousands of dollars)	80	81	83
Direct Man-Years			
Scientific	1.4	1.4	1.4
Others	4.1	4.2	4.3
Total	5.5	5.6	5.7

Background, Status and Future Plans:

Therapy of the acute radiation syndrome, utilizing whole blood transfusions, antibiotics, fluids, and intensive nursing care indicates that it is possible to save essentially all dogs exposed at X-ray dose levels approximately 1.6 times the LD_{50} dose.

The successful treatment of dogs receiving a potentially lethal dose of radiation thus provides an excellent approach to the study of regenerative capacity of hematopoietic tissues.

Studies on the Regenerative Capacity of Hematopoietic Tissue Following Supra-lethal whole body radiation in the Dog

E. P. Cronkite, V. P. Bond, E. Usenik

Treated dogs surviving 400 r acute total body radiation have been studied for at least 6 months. Regeneration of hematopoietic tissue was manifested by rising peripheral blood counts. Myelopoietic and erythropoietic regeneration in the bone marrow was often evident at 40 days post-exposure. Although the onset of regeneration of the different cell lineages appeared to be simultaneous, the rates of regeneration were not identical.

These studies are to be continued, to further demonstrate the regenerative capacity of the formed elements of the blood and to assess the long-term effects of dogs that have recovered from large doses of radiation.

PROPOSAL AND AUTHORIZATION
FOR RESEARCH OR DEVELOPMENT

NYO-149

1. Project Title: Radioisotopic Labeled Hormones to Determine Action Sites			2. Date: May 1959
3. Budget Activity No: 6120-8	4. Budget Item No:	5. Contractor's No:	6. Method and Time of Reporting Progress: Quarterly & Special Reports
7. Contractor: Associated Universities, Inc. Brookhaven National Laboratory		8. Working Location: Upton, New York	9. Contract No: At-30-2-GEN-16
10. Persons in Charge: I. Schwartz		11. Starting Date: Continuing	

	F. Y. 1959	F. Y. 1960	F. Y. 1961
Cost (in thousands of dollars)	70	71	72
Direct Man-Years			
Scientific	2.2	2.2	2.2
Others	2.8	2.9	3.0
Total	5.0	5.1	5.2

Background, Status and Future Plans:

The mechanism of action, particularly at the molecular level of homones remains obscure. The deficiency, excess, or qualitative abnormality of hormones is correlated with major disease processes such as diabetic ketosis, atherogenic changes in blood lipids, and diabetes insipidus secondary to radiation or other factors. The preparation of hormones labeled with low energy, alpha or beta-emitting, isotopes would make it possible to study, at the subcellular and molecular level, the crucial questions in endocrinology underlying these important clinical problems.

Mechanism of Hormone Action
I. Schwartz, A. Debons, C. Fong

The development of labeling procedures is planned in an attempt to obtain hormone preparations with high specific activity, double labels (tritium and sulfur-35 for example), without changing the biological activity of the hormone. Hormones of known chemical structure have been selected to begin these studies, vasopresin and oxytocin.

1177386

PROPOSAL AND AUTHORIZATION
FOR RESEARCH OR DEVELOPMENT

NYO-149

1. Project Title: Special Projects			2. Date: May 1959
3. Budget Activity No: 6120-9	4. Budget Item No:	5. Contractor's No:	6. Method and Time of Reporting Progress: Quarterly & Special Reports
7. Contractor: Associated Universities, Inc. Brookhaven National Laboratory		8. Working Location: Upton, New York	9. Contract No: AT-30-2-GEN-16
10. Persons in Charge: (See individual projects below)		11. Starting Date: Continuing	

	F. Y. 1959	F. Y. 1960	F. Y. 1961
Special Projects - Summary			
Cost (in thousands of dollars)	105	155	170
Direct Man-Years			
Scientific	1.9	1.9	1.9
Others	2.2	2.2	2.2
Total	4.1	4.1	4.1

These projects are summarized under the following headings:

1. Educational Conferences
2. Marshallese Survey
3. Summer Student Institute
4. Occupational Medicine Clinic

1. Educational Conferences
Persons in Charge: L. E. Farr and S. W. Lippincott

	F. Y. 1959	F. Y. 1960	F. Y. 1961
Cost (in thousands of dollars)	20	25	25
Direct Man-Years	-	-	--

Background, Status and Future Plans:

A stated educational effort of the Department is the organization of at least one special conference each year in conjunction with the Division of Biology and Medicine of the Atomic Energy Commission. These conferences are, in general, for educators to examine with active investigators specific responsibilities in the field of atomic medicine. The exact format of the conference is altered in each case to meet the specific needs of the group concerned.

The third of these conferences was held on December 15 and 16, 1958 to which all Deans of Medical Schools in the United States and Canada were invited to discuss, and hear discussed by BNL personnel and eminent authorities selected from various organizations, "The Impact of Atoms on Medical Science and Education". Approximately 75 per cent of the invitees attended and the conference was adjudged most successful.

(See continuation sheet)

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2. Marshallese Survey
Person in Charge: R. A. Conard

	F. Y. 1959	F. Y. 1960	F. Y. 1961
Cost (in thousands of dollars)	85	115	110
Direct Man-Years			
Scientific	1.9	1.9	1.9
Others	2.2	2.2	2.2
Total	4.1	4.1	4.1

Background, Status and Future Plans:

In March 1954 following the detonation of a nuclear device, 239 Marshallese people were accidentally irradiated with fallout. These people have been taken care of and studied initially and annually by medical teams under the auspices of the Division of Biology and Medicine of the Atomic Energy Commission. These studies are under the sponsorship of the Medical Department, Brookhaven National Laboratory and Dr. Robert A. Conard has been appointed by the Laboratory as chief of the Marshall Islands study project, and is in charge of organizing and carrying out the annual medical surveys of these people in the Marshall Islands. Much valuable information has been and is being obtained on the effects of fallout radiation on human beings which is being made available to the medical profession and scientific world through publications.

The five year post-exposure survey was carried out in February-March 1959 and has just been completed. The data from this survey is in the process of being analyzed. The results of the four year post-exposure survey completed in March 1958 are now being published in complete form under the title "Medical Survey of Rongelap People, March 1958, 4 Years After Exposure to Fallout" as BNL Report No. 534 (T-135). In addition this report in abbreviated form has been published in Radiation Research (Effects of Fallout Radiation on a Human Population) Proceedings of the International Congress of Radiation Research, Supplement 1, page 280, 1959.

Plans are now being made for the next annual medical survey to take place in March 1960 at 6 years post-exposure. Since the acute effects of the radiation exposure in these people have largely subsided, efforts in the future will be concentrated primarily on possible late effects of radiation and on the radiation ecological aspects of their living in a low level contaminated environment. In addition to the routine physical examinations and hematological work, emphasis will be placed on examinations for degenerative disease, carcinogenesis, aging effects and other late effects. Results of environmental contamination in the people will be studied by gamma ray spectroscopy of individuals using the steel room and radiochemical analyses of urine, food, soil, and marine samples. Studies of the radical homogeneity of these people and genetically inherited characteristics will be continued in view of their importance in interpretation of findings which may or may not be related to radiation effects. Participation in these surveys by scientists, medical schools, universities, and governmental organizations will be encouraged in order that desirable personnel may gain first-hand knowledge of radiation effects on human beings by studying these people. It is possible that future surveys may be reduced somewhat in scope when it is positive that the acute effects of exposure have unquestionably subsided. Possibly at some time in the future surveys on alternating years may be considered. However, at this time it is believed that annual surveys are indicated particularly since medical care and collection of data on the Islands ordinarily are not satisfactory and the project would suffer from extended intersurvey periods at this time.

3. Summer Student Institute
Persons in Charge: L. E. Farr and W. W. Shreeve

	F. Y. 1959	F. Y. 1960	F. Y. 1961
Cost (in thousands of dollars)	-0-	15	35
Direct Man-Years	-	-	-

Background, Status and Future Plans:

The role of the Brookhaven National Laboratory in medical education has been evaluated as follows:

(See continuation sheet)

3. Summer Student Institute (Continued)

BNL should center its major educational efforts upon physicians of faculty rank because thereby information is multiplied and young faculty members may, as the result of such experiences, elect a research-academic career as contrasted to a practice-academic career. This program must be carried out in such a fashion that for the exceptional student opportunities to participate in the work of the BNL medical program will remain open.

Careful study and consideration of the problems for several years has led to the conclusion that for medical students the most effective, appropriate and efficient extension of Brookhaven to undergraduate medical education would be accomplished by giving a highly modified version of a Medical School course at Brookhaven. By this means can the student be given a suitable and proper introduction to the broad problems of nuclear medicine from public health to patient care, from nuclear physics to disease prognosis. If, however, such a course were given by Brookhaven Staff alone it would divert BNL physicians from their basic research. Therefore, the course should be given by a Faculty assembled from Medical School Faculties, by men skilled in conducting teaching of medical students and by men interested in the field of atomic energy. The Faculty in turn will be Research Collaborators in the Medical Department participating with BNL scientists in research as do all other Research Collaborators in the Department. Thereby diversion is prevented and an actual reinforcement of program is attained. Since the course will be a responsibility of Faculty and it is the decision of the School to grant credit for the same, it is in exactly the same relationship to Brookhaven that the Ph.D. student is when he is doing his thesis research at Brookhaven. By control of course content, it can be assured that proper experiences are given ranging from laboratory manipulations of radioisotopes to waste disposal, hot laundry and personal physical examinations. By encouraging use of Brookhaven Medical Department personnel in lectures but limiting lectures by any given individual to two, the main reliance cannot be placed on BNL Staff yet their lectures will give the real impact to the course. Utilization of wards by the Faculty for student rounds including whole body counter usage will be wholesome for all. Participation should be by allotment to Medical Schools on some basis which will be established and which will be rotated in a manner to provide various medical schools with a chance to participate. Obviously some experience must be obtained to determine instrumentation needs, depth of course and hours required of the Faculty.

It is clear that no firm commitment should be made in regard to any new variant of the summer student program without experience that would indicate the value of the new approach. It is equally clear that such an experiment cannot be carried out jointly with 85 medical schools but that the best experimental approach is to find one medical school which is favorable to the idea and then to explore it for one year after which conclusions may be drawn and a course for further action plotted. The many details requiring agreement made it clear that if anything were to be accomplished during the summer of 1959, it must be done with a single medical school ready and willing to participate in such an educational experiment. Converstions held with Dr. John Truslow, Dean of the University of Texas, during the Conclave indicated a willingness to carry on the necessary experimental study. On February 4, 1959 in Galveston, Texas, this matter was further explored with Dean Truslow and members of his Faculty. On March 2, 1959, Dr. Robert Cooley, Professor and Chairman of the Department of Radiology, visited Brookhaven for additional conversations. The discussion was most enthusiastically received by the Texas Medical Faculty. A three man curricula committee from Texas visited Brookhaven March 30th and 31st to establish jointly with Brookhaven Medical Department members curriculum content. Brookhaven's facilities must be properly used and the high quality of all Brookhaven's activities must be maintained. A preliminary student selection has been made. Eight students have been chosen and three faculty members will be chosen for the first joint-BNL-Medical School Course in Principles of Nuclear Medicine to run June 28 to July 31, 1959 at Brookhaven.

(See continuation sheet)

4. Occupational Medicine Clinic
Persons in Charge: R. Love and M. Hagaman

The largest Laboratory-supported activity of the Medical Department is the Occupational Medicine Clinic. This organization provides to the Laboratory as a whole the necessary industrial medical services for proper operation. The work of the clinic has risen steadily with each year of the Laboratory's operation and the ultimate size of the operation cannot yet be determined. The total number of visits to the occupational medicine clinic during Fiscal 1958 was 10,692. Similarly, the total number of x-ray examinations increased from 1,780 to 2,005. Man-years and costs are not included above, but are part of the General and Administrative costs of the Laboratory and are distributed as Indirect Expense.

PROPOSAL AND AUTHORIZATION
FOR RESEARCH OR DEVELOPMENT

NYO-149

1. Project Title: Beneficial Applications of Atomic Energy - Cancer Research			2. Date: May, 1959
3. Budget Activity No: 6310	4. Budget Item No:	5. Contractor's No:	6. Method and Time of Reporting Progress: Quarterly & Special Reports
7. Contractor: Associated Universities, Inc. Brookhaven National Laboratory		8. Working Location: Upton, New York	9. Contract No: AT-30-2-GEN-16
10. Persons in Charge: L. E. Farr, M.D. (See individual project proposals)		11. Starting Date: Continuing	

SUMMARY

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Project Title: Cancer Research - 6310

17. Operating Costs (in thousands of dollars)

	Estimated 1959	Estimated 1960	Estimated 1961
Labor (including benefits)	273	406	477
Materials, Travel, etc.	61	100	112
Development Subcontracts, Special Proc.	7	6	6
Total Direct	341	512	595
Special Power	-0-	-0-	-0-
Reactor and/or Accelerator Usage	20	34	35
Technical Services (from BNL Service Units)	40	54	62
General & Administrative Overhead	179	250	258
Total	580	850	950

18. Plant & Equipment Directly Required (Shown here for information only)

	Estimated 1959	Estimated 1960	Estimated 1961
(A) Construction			
(B) Equipment (in thousands of dollars)	60	80	65

19. Direct Man Power

	Estimated 1959	Estimated 1960	Estimated 1961
No. of Man Years			
Scientists, Research Associates	9.5	13.0	15.0
Visiting Scientists	2.0	2.0	2.0
Scientists - Total	11.5	15.0	17.0
Technical	27.5	36.0	44.5
Administrative & Service	4.0	5.5	6.0
Total	43.0	56.5	67.5

20. Comments

1177392

PROPOSAL AND AUTHORIZATION
FOR RESEARCH OR DEVELOPMENT

NYO-149

1. Project Title: Neutron Capture Therapy			2. Date: May, 1959
3. Budget Activity No: 6310-1	4. Budget Item No:	5. Contractor's No:	6. Method and Time of Reporting Progress: Quarterly & Special Reports
7. Contractor: Associated Universities, Inc. Brookhaven National Laboratory	8. Working Location: Upton, New York		9. Contract No: AT-30-2-GEN-16
10. Persons in Charge: L. E. Farr, J. S. Robertson, E. E. Stickley	11. Starting Date: Continuing		

	F. Y. 1959	F. Y. 1960	F. Y. 1961
Cost (in thousands of dollars)	395	560	630
Direct Man-Years			
Scientific	9.2	12.5	14.0
Others	19.6	25.2	31.5
Total	28.8	37.7	45.5

Background, Status and Future Plans:

Neutron Capture Therapy (neutron distribution in tissue, pharmacology of target elements, test of tumor destruction, medical reactor technology, trial therapy and clinical evaluation, complementary studies with accelerators). In this research effort significant progress has been made in trial therapy of tumors of the central nervous system. In the course of these studies new aspects of pharmacology, as a scientific discipline have emerged as well as specific projects, such as particularly the toxicology of boron. With the new medical reactor, studies will be markedly extended on the clinical evaluation of neutron capture therapy for the control of intracranial neoplasms. Already explorations have been done looking toward the use of this procedure in control of neoplasia in other regions of the body, an advance made possible only by the new reactor. Studies of the effects of neutrons both thermal and epithermal on the central nervous system and other systems of the body will be continuing. Complementary studies of tissue radiation by particles derived from accelerators will be continued in experimental animals.

(a) Neutron Capture Therapy of Glioblastoma Multiforme
L. E. Farr, Y. L. Yamamoto, O. D. Easterday, E. E. Stickley
J. S. Robertson, S. W. Lippincott

Neutron capture therapy is an experimental radiation treatment which uses energetic heavy particles created inside the diseased state. It is based on localizing a suitable target, non-radioactive boron-10, in the intended site of treatment and then directing a stream of slow neutrons through that region. In the case of brain tumors, the phenomenon of the blood-brain barrier, by retarding the appearance of boron in normal brain tissue, enhances the selective kinetics, or favorable concentration ratio since the barrier does not exist in the tumor. For best effects the targe. element is so chosen that the decay of the excited isotope is immediate and results in energetic heavy particles.

The application of thermal neutron capture reactions to therapy of malignant tumors remains very attractive. Boron-10 as a capture element appears satisfactory for trials. The procedure is still complicated, and various alternative maneuvers must be tried to determine which is most efficacious.

Intra-arterial injections through the ipselateral internal carotid artery were used. Sodium pentaborate was used and a dose of 35 mg/kg seemed as effective as 50 mg/kg used previously. When the reactor loading was changed from natural to enriched uranium, the troublesome skin lesion problem previously eliminated returned although the flux remained the same. The first patient exploration in the Medical Research Reactor has occurred, but it is too early to give any results. Complete pathological studies will be done, and until

(See Continuation Sheet)

Neutron Capture Therapy of Glioblastoma Multiforme (Continued)

these are completed, definite conclusions cannot be made. These procedures will be continued during the coming year to obtain a comparative test of the Medical Research Reactor.

(b) Neutron Capture Therapy Effectiveness and Tissue Effects of Thermal Neutron Exposures.
L. E. Farr, Y. L. Yamamoto, S. W. Lippincott

Extensive histological studies are required in the exploration of this new therapeutic modality for not only must the efficacy of control of the tumor be established, but the harmlessness of the procedure in relation to all other central nervous system structures must be unequivocally demonstrated. Physiological and clinical studies will ultimately find their support in careful histological documentation. Extensive methodology development is simultaneously required. For full evaluation it is necessary to know both the alterations which may be encountered in central nervous system structures of so called normal man and the effects of a tumor on all other structures of the brain as well as for comparison of the effects of other therapeutic procedures which are used.

During the past year 19 brains have been embedded in celloidin and sectioned by the guillotine technique at levels of about 1 cm. Any possible effect of the neutron capture procedure in either the neoplasm or the remainder of the non-involved brains can thus be studied by the conjointed efforts of the neuroanatomists and pathologists participating in this study. In general, it is felt that the procedure has not damaged the normal tissues, whereas various alterations at the cellular level including the vasculature, as well as neoplastic cells, have occurred within some irradiated tumors as compared to control (comparative) neoplasms not treated in this manner. The evaluation and significance of these findings are now under consideration with particular reference to the natural history of the disease.

(c) Neutron Capture Therapy Procedures in Control of Tumor
L. E. Farr

A tumor induced through the use of methyl cholanthrene in the brain of the mouse is transplantable and is highly invasive. For seven years it has been used at BNL as a test tumor in the study of maneuvers for neutron capture therapy and the evaluation of certain concepts in this therapeutic procedure.

During the past year this transplantable tumor has been used both as an intracerebral transplant and as an intra-muscular transplant. Effectiveness of neutron exposure has been explored with variations in (1) time of exposure after boron injection, (2) total neutron exposure, (3) total dose of boron. This is in an attempt to determine the parameters which may be expected to be required for successful conclusion of patient therapy. Effectiveness does not parallel tumor-brain ratio. A tumor boron concentration is most effective in the period of 20 to 30 minutes post-injection. Total neutron exposure, 10^{11} per cm^2 is required throughout the tumor for a cure. A boron dose of 25 mg/kg gave a 96% cure and 50 mg/kg gave a 100% cure.

Thus, success has begun to appear. These findings suggest a possibility of achieving control with a smaller boron dose and of the same order as presently being given to patients. These studies will continue during the coming year.

(d) Medical Research Reactor Studies.
E. E. Stickley, S. Fine

Construction of the Medical Research Reactor has been completed and it has been brought into operation with the extensive help and cooperation of the BNL Nuclear Engineering and Reactor Operations Departments. The earliest phase of its use has included measurement of the emergent neutron flux and the characterization of the several radiation components present at the various treatment areas. There has also been continuing development of devices and accessories specifically adapted to treatment of patients and the parallel studies with small animals.

As of April, calibration tests were begun and on May 15 the first patient exploration was made. Full exploitation of the new and unique capabilities of the Medical Research Reactor will be undertaken. The work of first importance will be establishment of procedures and equipment to be used in the next series of neutron

(See Continuation Sheet)

Medical Research Reactor Studies. (Continued)

capture therapy experiments. Dosimetric analysis of tissue-equivalent phantoms will be carried out using techniques of foil activation, ionization chambers, and other detectors suitable for particular situations. Apparatus for producing and exploring the diagnostic and therapeutic possibilities of very short-lived radioactivities will be developed and improved. These will be used within the same patient and animal treatment rooms, holding delivery and application times to a minimum. The third major use of the MRR is in activation analysis; here it will become possible to emphasize even further the advantages of short-lived radioactivities by setting up the handling and analyzing equipment at the face of the reactor. The broad beam cell built to provide whole-body radiation for small animals is to be applied in studies of the hazards and general effects of the several constituents of reactor-originated and reactor-mediated radiation complexes.

(e) General Radiation Effects of Accelerator Generated Neutrons
J. L. Bateman and H. A. Johnson

Accelerator neutron studies have progressed through a full-scale pilot run using the 3 Mev Van de Graaff machine in the Physics Department. This is a unique experimental approach directed toward resolving the question of the comparative biological destruction caused by neutrons throughout the complete energy range. These studies correlate the results obtained from reactor neutrons, both in the fission spectrum and in the thermalized energy range, with those obtained from selected accelerator target reactions, contributing definite biological information of critical value as to the influence of neutrons in this previously unexplored energy region of presumed greatest hazard. Exposure holders for mice have been made to provide radiation in the fractional-megavolt energy range; the experiments included stressed and unstressed control populations as well as equivalent standard x-ray exposures. Biological end-points were spleen, thymus, testis, and whole-body weights, with additional studies on cell damage in the genetic material.

These studies are to be expanded during the next year.

(f) Toxicity and Pharmacology of Target Elements and Compounds
O. D. Easterday

The toxicity and pharmacology of the target elements and their parent compounds which are employed in the neutron capture therapy research have assumed an important position relative to the experimental investigations of this therapeutic procedure. The objective of the toxicological and pharmacological investigations is to study and evaluate new elements and compounds containing these elements employing the techniques and principles of this scientific discipline for possible use in the neutron capture therapy program. These studies are required for several reasons: (1) to determine if the element or compound has possibility for neutron capture therapy, (2) to determine the degree of safety associated with the new material, (3) to determine its general pharmacology and (4) to utilize pharmacological mechanism and principles to manipulate or direct the material to the desired locus.

Several of the accomplishments that may be cited are: the pharmacology and toxicity of a new compound, sodium pentaborate decahydrate, have been continued. The investigations of sodium pentaborate decahydrate have progressed to the point where expanded studies concerned with evaluating its effectiveness in tumor-bearing animals and in patients having intracranial neoplasms are currently in progress. Complexing agents other than d-glucose have been investigated and the data are currently being evaluated, a new series of compounds containing the target elements lithium and boron, are being studied toxicologically. Additional instrumentational developments were made on the remotely controlled injection equipment used to administer the neutron capture agent to the patients.

The objectives for the next fiscal year(s) will be as follows: to continue the toxicological and pharmacological evaluation of the lithium-boron-containing compounds. Further instrumentational developments on the injection equipment will be made as these are demanded by the employment of the medical research reactor in neutron capture therapy. New studies are planned which will be concerned with investigating the spectroscopy of the presently employed drugs and their complexes. To commence physical, chemical, toxicological and pharmacological studies on a new series of organic compounds containing the target element boron.

(See Continuation Sheet)

(g) Solubility of Organo-Boron Compounds
J. S. Robertson

In the exploration of the use of various boron compounds for neutron capture therapy, it is highly desirable to know the optimal ratio of the concentrations of the boron compounds and other substances in the solution to be injected. Phase system studies of the Na-borate-glucose-water system were conducted in the lower concentration region (up to 10 grams each of Na-borate and glucose in 100 ml of water). Investigation of the phase system for higher concentrations of the Na-borates and of other organo-boron compounds is proposed.

PROPOSAL AND AUTHORIZATION
FOR RESEARCH OR DEVELOPMENT

NYO-149

1. Project Title: Vitamin and Amino Acid Metabolism in Neoplasia and Normality			2. Date: May, 1959
3. Budget Activity No: 6310-2	4. Budget Item No:	5. Contractor's No:	6. Method and Time of Reporting Progress: Quarterly & Special Reports
7. Contractor: Associated Universities, Inc. Brookhaven National Laboratory		8. Working Location: Upton, New York	9. Contract No: AT-30-2-GEN-16
10. Persons in Charge: L. V. Hankes		11. Starting Date: Continuing	

	F. Y. 1959	F. Y. 1960	F. Y. 1961
Cost (In thousands of dollars)	26	27	30
Direct Man-Years			
Scientific	1.2	1.3	1.5
Others	1.0	1.0	1.0
Total	2.2	2.3	2.5

Background, Status and Future Plans:

There is evidence that the urinary levels of metabolites of certain vitamins may vary in several neoplastic diseases. The metabolites of tryptophan are examples. Further, it has been suggested that certain of these metabolites possess carcinogenic activity. The study of the metabolism of tryptophan to niacin would be advanced by the synthesis of H^3 and C^{14} labeled intermediates so that the metabolism of tryptophan could be better understood, in health and disease and the possible carcinogenic activity of the compounds tested in animal experiments. Also, the origin and nature of compounds found in the urine of patients with certain diseases could be studied better if labeled compounds were available, specifically, the pentose sugar found in pentosuria.

(a) Metabolism of Tryptophan
L. V. Hankes

A metabolic derivative of tryptophan is a vitamin, niacin, while other metabolites are thought to be carcinogens. The study of tryptophan metabolism requires the synthesis of tritium or C^{14} labeled intermediates of tryptophan--niacin metabolism. Some of these compounds have been synthesized with the aid of collaborators and these include: kynurenine-4-C^{14}, aminoacetophenone-2-C^{14}, anthranilic acid-1-C^{14}, carboxyl-C^{14}-labeled-anthranilic acid, 3-hydroxyanthranilic acid-1-C^{14}-labeled 3-hydroxyanthranilic acid, and 5-hydroxyanthranilic acid-1-C^{14}.

During the present year metabolism studies were conducted with 3-hydroxyanthranilic acid-1-C^{14}, carboxyl-labeled-3-hydroxyanthranilic acid and 5-hydroxyanthranilic acid-1-C^{14}. The quantities of these compounds synthesized were very limited, and in quantities only large enough for animal studies. Techniques were developed for labeling tryptophan and kynurenine with tritium in large quantities for use in human neoplastic disease studies. The synthesis of tryptophan-5-C^{14} was started and this material will be used in patient studies. The synthesis of para-aminobenzoic acid-1-C^{14} was concluded and plans made for the study of its metabolism. Studies were continued on the mechanism of converting 3-hydroxyanthranilic acid into the vitamin, niacin. In the process of these rat metabolism studies it was discovered that the number one carbon of 3-hydroxyanthranilic acid becomes the number two carbon of acetate. This is a previously unknown source of acetate in the body. The C^{14} of 5-hydroxyanthranilic acid-1-C^{14} was found to be rapidly and quantitatively excreted in urine.

As larger quantities of these materials becomes available, the metabolism of these compounds will be studied in brain tumor patients pre- and post-boron, neutron treatment.

(See Continuation Sheet)

1177397

(b) Studies of Pentosuria
L. V. Hankes

The nature and source of the pento sugar appearing in urine of patients with pentosuria is unknown. C^{14} labeled-I-inositol provided by a collaborator will be administered to a patient with this disease and the urine will be studied for metabolites for the administered compound.

PROPOSAL AND AUTHORIZATION
FOR RESEARCH OR DEVELOPMENT

NYO-149

1. Project Title: Labeled Proteins for Metabolic Observation in Cancer Evaluation			2. Date: May, 1959
3. Budget Activity No: 6310-3	4. Budget Item No:	5. Contractor's No:	6. Method and Time of Reporting Progress: Quarterly & Special Reports
7. Contractor: Associated Universities, Inc. Brookhaven National Laboratory		8. Working Location: Upton, New York	9. Contract No: AT-30-2-GEN-16
10. Persons in Charge: S. W. Lippincott		11. Starting Date: Continuing	

	F. Y. 1959	F. Y. 1960	F. Y. 1961
Cost (in thousands of dollars)	159	263	290
Direct Man-Years			
Scientific	1.1	1.2	1.5
Others	10.9	15.3	18.0
Total	12.0	16.5	19.5

Background, Status and Future Plans:

A study has been under way to determine the physico-chemical properties of the serum proteins in neoplastic diseases. Principal approaches have included the following to study the nature of albumin and globulins: immunochemical procedures, electrophoresis, ultra-centrifugation, infrared spectroscopy, and physiological studies on the rate of disappearance of these proteins when labeled with radio-isotopes. The latter procedure makes it possible to determine the rate of degradation and/or synthesis of the specific protein to be studied. The clinical aspects of this investigation are currently being pursued.

The objective of this research is to determine whether patients with various neoplastic diseases have the same or different rates of turnover for normal I^{131} labeled proteins and for those obtained from cancer patients. If in man a difference in turnover rate between normal protein and a protein prepared from the cancer pateint could be detected, a laboratory test might be designed that would be capable of helping the physician to detect the individual who has cancer. Since at present there is no blood test which is capable of detecting cancer in the early stages, such a test if it can be developed, would be of immediate usefulness in establishing the diagnosis of cancer.

A total of 65 cases have been studied or are now in the process of being studied. The biological half-life of labeled globulins prepared from sera of normal subjects and from sera of patients with neoplastic diseases has been determined in patients with carcinoma of the breast and in patients with multiple myeloma. In the latter disease it has been observed that the turnover of globulins in patients with a beta type serum electrophoretic patterm is much slower than in those patients with a gamma type peak. The purpose of this study was to determine whether patients with breast carcinoma identify by turnover a difference between gamma globulins prepared from normal individuals and that prepared from patients with carcinoma of the breast, prostate, or bowel and multiple myeloma. In general, the turnover for the globulins from normal, breast, bowel, and prostate sources was slower than from the globulin from myeloma patients. These clinical studies are to be extended to 150 cases to ascertain the possible chemical applications of these observations.

It is to be emphasized that these patients are extremely ill and thus require extensive nursing care. For example, in those patients with metastatic breast cancer, spontaneous bone fractures occurred, and this then necessitates special nursing care. Extreme care is taken in establishing the diagnosis or in the case of normal individuals, the lack of a disease. These procedures require extensive laboratory tests. The minimum length of time that it takes to study one patient is approximately three months, although when multiple turnovers are done, this time period has been extended to as long as fourteen months. Thus in studies of this type, the amount of hospital care and service is extensive.

1177399

PROPOSAL AND AUTHORIZATION
FOR RESEARCH OR DEVELOPMENT

NYO-149

1. Project Title: Beneficial Applications of Atomic Energy - Medical Research			2. Date: May, 1959
3. Budget Activity No: 6320	4. Budget Item No:	5. Contractor's No:	6. Method and Time of Reporting Progress: Quarterly & Special Reports
7. Contractor: Associated Universities, Inc. Brookhaven National Laboratory		8. Working Location: Upton, New York	9. Contract No: AT-30-2-GEN-16
10. Persons in Charge: L. E. Farr, M.D. (see individual project proposals)		11. Starting Date: Continuing	

SUMMARY

1177400

Project Title : Medical Research - 6320

17. Operating Costs (in thousands of dollars)	Estimated 1959	Estimated 1960	Estimated 1961
Labor (including benefits)	188	239	311
Materials, Travel, etc.	41	59	73
Development Subcontracts, Special Proc.	5	3	4
Total Direct	234	301	388
Special Power	-0-	-0-	-0-
Reactor and/or Accelerator Usage	14	20	23
Technical Services (from BNL Service Units)	29	32	41
General & Administrative Overhead	123	147	168
Total	400	500	620

18. Plant & Equipment Directly Required
(Shown here for information only)

	Estimated 1959	Estimated 1960	Estimated 1961
(A) Construction			
(B) Equipment (In thousands of dollars)	20	25	25

19. Direct Man Power	Estimated 1959	Estimated 1960	Estimated 1961
No. of Man Years			
Scientists, Research Associates	6.5	8.0	10.0
Visiting Scientists	1.0	1.0	1.0
Scientists - Total	7.5	9.0	11.0
Technical	19.5	22.5	29.5
Administrative & Service	2.5	3.5	3.5
Total	29.5	35.0	44.0

20. Comments

1177401

PROPOSAL AND AUTHORIZATION
FOR RESEARCH OR DEVELOPMENT

NYO-149

1. Project Title: Maps of Metal Pathways with Especial Reference to Trace Metals and Central Nervous System Diseases			2. Date: May, 1959
3. Budget Activity No: 6320-1	4. Budget Item No:	5. Contractor's No:	6. Method and Time of Reporting Progress: Quarterly & Special Reports
7. Contractor: Associated Universities, Inc. Brookhaven National Laboratory		8. Working Location: Upton, New York	9. Contract No: AT-30-2-GEN-16
10. Persons in Charge: L. K. Dahl, G. C. Cotzias		11. Starting Date: Continuing	

	F. Y. 1959	F.Y. 1960	F.Y. 1961
Cost (in thousands of dollars)	360	450	550
Direct Man-Years			
Scientific	6.1	7.4	9.0
Others	20.2	23.8	30.0
Total	26.3	31.2	39.0

Background, Status and Future Plans:

The pathways of distribution, the site of primary and secondary accumulation, the factors affecting partition, and the regulation of total body content of metals, particularly trace metals, in health and disease is the concern of this study. These data are of the greatest importance in the development of diagnostic procedures and understanding of significance of measures designed to influence specific metals in the body. Emphasis is placed on the short half-life isotopes permitting precise physical studies. Manganese and sodium have been chosen as the basic reference metals since their differing distribution in various body systems and compounds.

(a) Maps of Metal Pathways with Reference to Central Nervous System Diseases
G. C. Cotzias, D. C. Borg, and A. J. Bertinchamps

Animal data show that trace metal deficiency, chiefly manganese, causes ataxia. Human data show that manganese poisoning results in Parkinsonian-like syndromes. Investigations include:

1. Analyses of tissues and tissue particulates for trace metals and their artificial isotopes. Brain is a main tissue of study.

2. Total body radioactivity studies of animals following introduction into the body of artificial radioisotopes of trace metals.

3. Studies of biliary excretion of manganese metals in animals, since this is the main excretory route.

4. Total body and regional turnover studies in humans divided into the following groups: Control, Parkinson's disease, Wilson's disease and Friedreich's ataxia.

The following findings have been noted:

A. The fast blood disappearance of injected manganese salts was correlated with transcapillary movement and entraced into the mitochondria.

B. The manganese pathway through the body was found to be specific for manganese but the influence of technetium has not been tested as yet. Iron is not confused with manganese and vice-versa.

C. A porphyrin containing manganese, rather than iron, was discovered in humans and was demonstrated in other mammals as well. This prophyrin seems associated with alkali-resistant hemoglobulin fractions and seems, therefore,

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to belong to the fetal group of pigments.

D. The excretion of manganese was found to be biliary. The excretion pattern is markedly different in normalcy versus manganese-overloads. When used as a bioassay, this pattern shows that many feeds are overloaded with manganese.

E. The transport mechanism for manganese in human plasma involves a B_1 globulin, most probably transferrin. Transferrin does not confuse iron with manganese. An interesting polymerization of transferrin was discovered which regulates the release of manganese.

F. Zinc was studied relative to A, B, C, and D. No zinc hemoglobulin was found as yet.

G. Metabolic patterns giving the impression of specificity were encountered in the above patients. Manganese seems to be involved with the clinical picture of these diseases, although precise interpretation of the findings is not possible at this time.

Emphasis will be given to clinical studies as the above program is continued

(b) Relationship Between Sodium and Hypertension
L. K. Dahl, W. Gordon, Jr., and L. Silver

Sodium as the major circulating ion of physiological importance,,as contrasted to potassium, provides accessibility lending itself well to a general reference metal. Further, despite the fact that salt has been a highly prized part of man's diet, its effects if used in great excess or in minute quantities are unknown. Without radioactive isotopes it was impossible to accurately and readily determine changes in biochemical lability of specific body components.

The following observations have been made: In a study of 1200 Japanese farm-laborers near Hiroshima, made by Brookhaven staff with the help of the Atomic Bomb Casualty Commission, the following data were accumulated: (1) about 20% incidence of hypertension; (2) remarkably little evidence of atherosclerosis both clinically and by study of chest x-rays, EKG's and serum lipids; (3) a daily salt (NaCl) intake of about 11-15 gm. It was concluded that atherosclerosis is not the necessary complication of hypertension which it is assumed to be in Western societies.

Observations on the respiration of rat aorta at different ages in both sexes were concluded. Studies on ion-binding by connective tissue continued, as well as of magnesium metabolism with Mg^{28} in humans and hypertensive animals. Studies on long term effects of salt feeding in dogs and rats are continuing.

Sodium turnover will be studied in patients with and without hypertension using Na^{22} and the whole body counter under a variety of stresses. Similar studies in rats will be continued. In view of known effect of antidiuretic hormone (ADH) on blood pressure and electrolyte excretion, it is planned to explore ADH metabolism in patients and animals with and without hypertension. Activation Analysis of metals in organs and blood vessels will be continued. Studies on ion-binding by connective tissue will be continued. Studies on long-term effects of salt feeding will be continued.on dogs, rabbits, and rats.

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