PARTIAL DOCUMENT

725513

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory — Laboratory

06-Biology & Medicine — Program

1. Contractor: Associated Universities, Inc.
Contract No.: AT-30-2-GEN-16
Task No.:

2. Project Title: Effects of Radiation on Living Organisms--Medical Research
189 No.: 06-3

3. Budget Activity No.: 06-01-01

4. Date Prepared: May 1970

5. Method of Reporting: See sub-activities

6. Working Location: Brookhaven National Laboratory

7. Person in Charge: See sub-activities

Principal Investigator: See sub-activities

8. Project Term: Continuing
From: To:

PRIVACY ACT MATERIAL REMOVED

9. Man-Years:	FY 1970	FY 1971	FY 1972
Sci., Res. Assoc. (Ph.D. or Equiv.)	6.5	3.5	5.5
Visiting Sci.	-	-	-
Prof. (B.S. or Equiv.)	-	-	-
Sci. & Eng. - Total	6.5	3.5	5.5
Technical	21.0	20.0	25.0
Adm. & Service	4.0	4.0	4.0
Guests & Research Collaborators	6.0	6.0	7.5
Total	37.5	33.5	42.0

10. Costs (In Thousands of Dollars):	FY 1970	FY 1971	FY 1972
Labor (including benefits)	407	355	471
Mats., Trav., Dev. Subcont., Spec'l Proc.	78	65	85
Reactor, Accel., and/or Computer Usage	14	14	16
Allocated Technical Services	17	15	20
Gen. & Adm. Overhead			
Total Research Cost			

PRIVACY ACT MATERIAL REMOVED

11. Reactor Concept:

12. Materials:

1178733

Project Title: Effects of Radiation on Living Organisms--Medical Research 06-01-01

SUMMARY

Sub-Activity	Title
	Medical Research
06-01-01-(a)	Early and Late Effects of Radiation of Different Quality and at Different Dose Rates
06-01-01-(b)	Mechanisms and Radiosensitivity of Immune Reactions

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory — Laboratory

06-Biology & Medicine — Program

1. Contractor: Associated Universities, Inc.
Contract No.: AT-30-2-GEN-16
Task No.:

2. Project Title:
Effects of Radiation on Living Organisms-Medical Research
Early and Late Effects of Radiation of Different Quality and at Different Dose Rates
189 No.: 06-5

3. Budget Activity No.: 06-01-01-(a)

4. Date Prepared: May 1970

5. Method of Reporting
BNL Annual Report
BNL Monthly Letter to AEC
Bulletin of the Medical Department

6. Working Location:
Brookhaven National Laboratory

7. Person in Charge:
V. P. Bond
H. H. Rossi
C. J. Shellabarger
Principal Investigator:
V. P. Bond — C. J. Shellabarger
H. H. Rossi — A. L. Carsten
J. L. Bateman

8. Project Term:
Continuing
From: To:

9. Man-Years:

Direct Man-Years	FY 1970	FY 1971	FY 1972
Scientific & Professional	3.1	2.5	3.5
Other	14.9	16.0	18.9
Guests & Res. Collaborators	6.0	5.0	6.5
Total	24.0	23.5	28.9

10. Costs (In Thousands of Dollars):

	FY 1970	FY 1971	FY 1972
Research Division	396	407	535
Hospital Division	39	37	25
Research Costs	435	444	560

11. Reactor Concept:

12. Materials:

Project Title: Effects of Radiation on Living Organisms - Medical Research
Early and Late Effects of Radiation of Different Quality and at Different Dose Rates 06-01-01-(a)

13. Publications:

Shellabarger, C. J., Bond, V. P., Cronkite, E., Aponte, E. The relationship of dose of total body ^{60}Co radiation to incidence of mammary neoplasia in female rats. Radiation-induced Cancer (Proc. IAEA Symposium, Athens, Greece, April 1969), pp. 161-72, IAEA, Vienna, 1969.

Bengtsson, L., Robertson, J. S., Goodman, L., and Marino, S. Computer evaluation of monoenergetic neutron irradiations in radiobiology. Health Phys. (in press).

Taketa, S. T., Carsten, A. L., Cohn, S. H., Atkins, H. L. and Bond, V.P. Active bone marrow distribution in the monkey. Life Sci. (in press).

Bengtsson, L. G., Robertson, J. S., Goodman, L. J. and Marino, S. A. ANY. A computer program for the calculation of parameters of dosimetric significance in monoenergetic neutron irradiations. Informal Report BNL 13551.

Carsten, A. L., Caveness, W. F., Roizin, L., and Machek, J. Bilateral depression in photo-evoked response as a late effect of unilateral visual cortex x-irradiation. Brain Research (in press).

Montour, J. L., Straub, R. F. and Shellabarger, C. J. The relative biological effectiveness of 2.2 GeV protons for thymic and splenic weight loss in mice. Intern. J. Rad. Biol. 15, No. 5, 491-6 (1969).

Bateman, J. L. and Snead, M. R. Current research in neutron RBE in mouse lens opacity. Presented at the Symposium on Neutrons in Radiobiology, Oak Ridge, Tennessee, November 1969.

14. Scope:

The primary objectives in these studies are the accumulation of experimental information for the development and evaluation of theories of radiobiologic action through systematic collection of quantitative data on the effects of irradiation on biological tissue including the role of radiation in inducing cancer. A major research tool in these studies is the Van de Graaff generator modified from its previous pulsed operation with the BNL Cosmotron to provide a steady source of monoenergetic neutrons.

(Bond, Rossi)

Lens opacification studies in the mouse have revealed a significantly increased incidence of minute discrete posterior subcapsular opacities with increasing age or following irradiation. At this very low level of biological effect, radiation effectiveness has shown a strong dependence on radiation quality; relative biological effectiveness (RBE) values above 100 have been demonstrated when neutrons of 0.043 Mev were compared to 250 kVp x rays. To ascertain whether similar minute changes occurred and

14. Scope: (Cont'd.)

could be measured in man, a workable method of examination and scoring was developed utilizing BNL Laboratory employees exposed to very low levels of ionizing radiation and metastatic cancer patients maintained on cytotoxic drugs.

Prior studies with several different proliferative cell systems in the mouse, designed to assay the influence of radiation quality and dose rate upon radiation effectiveness, also confirmed a dependence of the type of dose-effect upon radiation quality. This phenomenon means that the relative efficiency of two different radiations will vary according to the dose magnitude at which they are compared. Adequate documentation of these relations, however, requires a biological system capable of simple response over broad ranges of dose, particularly at low doses where the radiation differences are greatest. The mammalian optic lens offers such a system, and is being utilized in investigations of wide variation in biological and physical (radiation) factors. This highly sensitive criterion of radiation effectiveness embodies an "isolated" proliferative cell system in which progeny of the continuously dividing epithelial germinal cells mature into single lens fibers and incorporate into the transparent lens body. Visible single fiber defects constitute evidence of earlier injury to the epithelial germinal cells. Multiple defective fibers appear to derive from each injured cell, causing an amplified biological manifestation. These studies constitute a portion of the investigational activity of the joint Columbia University-Brookhaven National Laboratory radiation research program. Monoenergetic fast neutrons of discrete energies in the fractional to few Mev range, produced by the p-T, d-T, and d-D reactions on a 3 Mev Van de Graaff generator, have been essential to these studies, as have been the correlated dosimetry and quality measurements carried out by the Columbia University Radiological Research Laboratory. (Bateman)

The objective of another series of studies in the joint Columbia-BNL program is a better understanding of the nature of radiation-induced chromosome lesions. The fruit fly, Drosophila melanogaster is exposed to x rays and neutrons in these studies. (Gonzalez)

Several collaborative studies are carried on to investigate the effects of x irradiation on the central nervous system. In studies on the effects of x irradiation of the rat forebrain on learning and morphology (in collaboration with E. Ingersoll, Medical College of Virginia), the rat forebrain is x irradiated, and the rat's learning ability tested at various times post-irradiation and following various exposures. Post-irradiation time periods out to 12 months are being investigated with exposures from 2,500 to 5,000 rads. The "delayed maze running ability" is used to evaluate loss of mental function. Complete light and electron microscopy is carried out on all animals. A correlation of loss in function and architecture is the desired end result. The body of data relating radiation

14. Scope: (Cont'd.)

dose and time post exposure to decrement in maze running ability and changes in morphology is incorporated with the results in other species to develop a better understanding of the pathogenesis of the CNS radiation lesion in mammals receiving exposures in the therapeutic range. Also, a further insight into structure-function relationships in the CNS is sought.

Comparative study of the effects of x irradiation on the elasmobranch brain is carried on with I. Klatzo, NINDS. The differing morphology of the elasmobranch as compared to the mammalian (location of astrocytes grouped in intimate contact with vessels in sharks as opposed to scattered at greater distances in mammals), the importance of the astrocyte to brain metabolism and as a first indicator of radiation damage, and the unusual resistance of the shark brain to other injury (particularly to alterations in blood brain barrier) have led to an interest in studying radiation effects in this species. Sharks are irradiated with doses in the 1,000 to 30,000 R range, limited to specific brain areas, and serially sacrificed for histochemical and electron microscopic evaluation. Alterations in blood brain barrier are evaluated using standard tracer and dye techniques, as well as the newer peroxidase method.

The effects of radiation on the monkey brain are studied in collaboration with W. F. Caveness, NINDS; L. Roizin, Columbia University; and D. Farrar and E. S. Graham, Holloman Air Force Base. Monkeys, age 12 weeks to 4 years, received whole and partial brain x-ray exposures to the hand-face area and visual cortex in single and multiple doses ranging from 150 to 67,000 rads. Clinical and EEG evaluation are combined with serial sacrifice at post-irradiation periods from 24 hours to 96 weeks. Normal histological procedures and electron microscopy are combined with special techniques of statistical evaluation of alterations in the dendritic plexus and synaptic area to correlate changes in function as measured by the EEG. Special techniques of frequency analysis of photoevoked response are used in the EEG analysis. In addition, the loss in visual acuity in trained animals is measured following x irradiation of both visual cortices. Special surgical techniques are employed to separate the function of each hemisphere, in order to study their separate and related reactions to injury. These studies are designed to provide information on the relationship of radiation dose to the pathogenesis of radiation lesions and loss of function. There is particular interest in the measurement of the dose threshold for decrements in visual acuity and reduction in photoevoked response. The split brain technique combined with the induction of radiation necrosis in limited brain areas, should lead to a better understanding of intrahemispheric stimulatory and inhibitory phenomena in the monkey. The measurement of decrement in vision related to radiation dose should lead to a better understanding of what might be considered safe threshold levels for the exposure of normal brain tissue in radiotherapy.
(Carsten)

The long range objective of a major study in this activity is to understand the mechanisms of radiation carcinogenesis. It seems unlikely

(See Continuation Sheet)

14. Scope: (Cont'd.)

that carcinogenetic mechanisms will be the same for all organs in all animals. Therefore, it seems reasonable to select certain organ systems in certain animals for study in the hope that as these model systems are understood the knowledge may be applied to problems of tumor development in man. The system to be studied in greatest detail is the relatively large and relatively rapid neoplastic response of rat mammary tissue to ionzing radiation. This particular animal model has been selected for the following reasons: there is now a large body of basic data on this system; BNL has had considerable experience with the system; it is a relatively "clean" experimental system that lends itself to meaningful experimentation; and the rat model system appears to be rather closely related to the human situation. Thus data acquired may be interpreted in terms of an academic interest in carcinogenesis and may be applicable to man. A series of experiments have been designed to achieve the short range objective of learning how changes in dose-rate are related to the magnitude of neoplastic response, and what the relative biological effectiveness of neutrons might be for this neoplastic response. Collaborating in these studies are: A.C. Upton at SUNY, on both the design and interpretation of experiments and the interpretation of pathological specimens; J.L. Montour, Medical College of Virginia, on a series of experiments dealing with the relative biological effectiveness of charged particles of varying energies; and L. J. Goodman, Columbia University, on both the production and measurement of neutrons, and the design of neutron experiments. (Shellabarger)

15. Relationship to Other Projects:

Related studies on minute discrete subcapsular opacities include opthalmological studies of Hiroshima and Nagasaki survivors. Related studies on ionizing radiation utilizing monoenergetic fast neutrons centered at BNL include those of Smith with Zea maize, and of Sparrow and Underbrink with Tradescantia occidentalis, both in the Biology Department. Elsewhere, lens opacification studies are conducted by Christenberg et al at ORNL and Riley et al at University of Iowa, but generally do not employ monoenergetic neutrons; Legeay at CEN Fontenay-aux-Roses used rabbits exposed to neutrons of 14 Mev. None of these quantitate the minute effects produced by very small doses of radiation at which RBE values are likely to be maximal. Related studies by Barendson and Broerse at TNO Rijswijk, Holland; Dennis and Boot at AERE, Harwell; and Berry at Churchill Hospital, England, use monoenergetic neutrons but generally are exploring higher dose ranges and particularly attempt to relate oxygen enhancement to radiation quality. (Bateman)

Related chromosome aberration studies are carried on by Savage and Neary at Harwell; Wolff and Heddle at the University of California at San Francisco; and Bender at ORNL. (Gonzalez)

Related studies on the effects of radiation on the central nervous system include Wolfgang at Walter Reed Army Hospital; Bruner, Lovelace

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

Foundation; Barnes, USAF School of Aerospace Medicine. (Carsten)

The following work is related to the radiocarcinogenesis studies: H. H. Vogel, Jr., University of Tennessee, has a project concerned with rat mammary carcinogenesis and neutron irradiation. Duplication of some aspects is inevitable. The BNL program is somewhat broader in scope because it is concerned with dose-rate effects and has the only wide range monoenergetic neutron source. Yu. I. Moskalev, Biophysics Institute, Moscow, has used the rat mammary tumor system; however, this program seems confined to only proton irradiation. N. C. Telles, Bureau of Radiological Health, USPHS, has a program of radiation-ethionine mammary carcinogenesis that is in part an outgrowth of the BNL study but will concentrate on the interaction of ethionine and x irradiation and thus will not overlap the work here. Charles Huggins, University of Chicago, and T. L. Dao, Roswell Park, have programs on chemical carcinogenesis using the rat mammary system but these are confined largely to studies of chemical carcinogens. L. H. Hempelmann, Rochester, has a program designed to study the possible association of radiation and breast cancer in man; however, this program appears to be confined to surveys of hospital records and does not include animal experiments. (Shellabarger)

16. Technical Progress in FY 1970:

Slit-lamp biomicroscopic examinations were made of unexposed residents of the Marshall Islands (Pacific Trust Territory) and of Marshallese persons exposed to fallout from the nuclear detonation at Eniewetok in 1954. The study included 80 controls and 41 persons who had been exposed to 175 R of whole-body gamma rays. (Smaller and statistically unusable numbers of persons exposed to 14 R or 70 R were also examined). The unexposed of 15 years of age revealed an average of 5 minute discrete opacities each, but this value rose steadily with increasing age to a maximum of 25 at 50 years of age, after which a plateau or slight decline occurred. The preceding extremes were found different at a confidence level between 65% and 95%. Persons exposed to 175 R yielded opacity counts slightly above their age-specific counterparts at the lower end of the age span, then rose rapidly with increasing age to a maximum of almost thirty opacities per person at the age of 27-33. Above this age, persons exposed to 175 R had opacity counts no greater than their age specific unexposed counterparts. The maximum difference between the two groups was at the age of about 30, at which time the opacity counts were different at about the 65% level of confidence.

The study of murine lens radiosensitivity to 250 kVp x rays as a function of age at exposure revealed a maximum occurring at three to four months of age preceded by a sharp rise from time of birth, and followed by a more gradual decline out to twelve months, the greatest age tested. The sensitivity was about four times greater at the maximum than at either extreme of age.

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16. Technical Progress in FY 1970: (Cont'd)

An experiment designed to examine reduction in opacification effect with dose fractionation at graded levels of total dose showed similar results at total single doses of 400, 200, 40 and 20 rads of 250 kVp x rays. In each case, the single exposure was most effective, and in several instances was more effective than a half dose followed at 24 hours by an exposure equal to the single dose. This presents strong evidence that the first dose has caused the lens epithelium to be considerably less sensitive, possibly by a synchronizing influence that causes a large portion of the cells to be in a radioresistant stage of their cycle at the time of the second exposure. A corollary likelihood would be that with equal split doses, the contribution of the two toward the net effect would be dissimilar, making a meaningful quantitative analysis, at the least, complex. (Bateman)

The x ray and 0.68 Mev experiments on Drosophila melanogaster were completed. Translocations increase linearly with increasing neutron dose indicating that the two chromosome lesions that are required for a translocation are caused by the same track. Translocations induced by x rays increase with a power of the dose greater than one, indicating that the two lesions are produced by independent tracks. The RBE varies from 4.0 at a dose sufficient to produce 1% translocations to 2.2 at a dose sufficient to produce 10% translocations.

Both x-ray-induced and neutron-induced recessive lethal mutations increase linearly with dose. Most of these are point mutations or small deletions. The RBE is 1.8. A typical semi-logarithmic survival curve was found for neutrons. The x-ray curves contain a slight shoulder. Although these data have not yet been analyzed, the RBE for the straight portion of both curves is about 3.6 During these studies it was observed that cycloheximide had no effect on egg hatching and data were obtained on the effect of maternal genotype on egg hatching. (Gonzalez)

Three additional experiments were carried out in which rats received forebrain exposures ranging from 2,500 to 5,000 rads and were tested for "delayed maze running ability" at 8, 12, 16, and 32 weeks post irradiation. In contrast to the earlier studies where the observation periods were only 4 weeks post exposure and the significant decrement in maze learning ability was evident, the decrement was observed in only a few animals at the 8 and 12 week periods and preliminary results indicate no decrement in learning ability in the 16 week and 32 week post irradiation period.

The collaborative work on the radiation effects on the elasmobranch brain were similarly curtailed due to a lack of funds available for overseas travel which is a necessary part of this study. However, some interesting results were obtained from the single series of experiments done at the Lerner Marine Laboratory, Bimini. Comparative studies in which rats, cats and sharks were given identical exposures to the forebrain and cerebellum indicated the marked species differences which had

16. Technical Progress in FY 1970: (Cont'd.)

previously been suspected. Whereas doses of above 5,000 rads caused marked radiation necrosis in the cats and rats, the examination of sharks as much as 14 months after exposure to doses of 5,000 to 10,000 rads indicated no observable effect on the shark brain with the exception of a small rectangular pigmented area on the shark's skin at the point where the radiation beam entered the tissue. An additional series of sharks were exposed for later sacrifice. These animals will be used for examination of the integrity of the blood brain barrier at long periods following exposure.

The complete examination of functional and structural change in monkeys receiving 3,500 rads to the right visual cortex was completed. As reported for preliminary results, it was found that these animals developed a bilateral depression in photoevoked response beginning at approximately 55 weeks post exposure and continuing out to at least 144 weeks post exposure. This finding was made even more intriguing by the lack of any demonstrable pathology in tissues on the unirradiated side. This lack of a structural functional correlate led to new studies recently initiated involving the surgical sectioning of the corpus callosum and optic chiasm in monkeys previous to unilateral exposure of the visual cortex. This split brain preparation should provide further information concerning the possible functional mechanism by which the abscopal depression in evoked response is caused. The animals with bilateral irradiation of the visual cortices were tested for decrements in visual acuity and preliminary results were confirmed in that all animals begin to develop a decrement in visual acuity as early as 12 to 14 weeks post irradiation and within a period of a few weeks thereafter became functionally blind (Carsten)

Dosimetry on a newly constructed Co-60 irradiator was completed with a resulting dose-rate of approximately 2 R/hr. This irradiator is now being used to study the effect this low dose-rate has on rat mammary neoplasia. Dosimetry on low-level Cs-137 sources available in the Biology Department was not done for two reasons; apparently adequate dosimetry will be done by others, and it does not now appear useful to go on to even lower dose-rates that can be obtained with the Biology facilities until results are in hand at the dose-rate of 2 R/hr. Dosimetry at the RARAF neutron source will be more than adequately done by the RARAF group by the time actual exposures of rats are begun.

Preliminary data on the effect of age at the time of exposure on incidence of mammary neoplasia were collected and it appears that animals exposed at 24 days of age are less sensitive than those exposed at 42 or 84, or 225 days of age. If confirmed, this age dependency will have a practical application, i.e., age at time of exposure must be controlled in each experiment, and data collected from animals at differing ages at time of exposures cannot be compared directly. Also, it is often stated that radiation sensitivity increased with decreasing age. Since the reverse appears to be the case in this experiment, an additional experiment may be designed, using a partial body irradiation technique to shield the

16. Technical Progress in FY 1970: (Cont'd.)

ovaries, in order to test the concept that the lowered mammary neoplastic response in young rats may be due to an increased radiation sensitivity of the young ovary.

A short-term fractionation experiment was completed. Four hundred R given in two equal fractions at intervals of 0, 2, 6, or 24 hours produced incidences of mammary neoplasia that were not different among the 4 irradiated groups. This means that an "Elkind-effect" or repair of sublethal radiation injury was either not present or too small to detect. Since cell killing and sublethal repair mechanisms have been implicated as being important in radiation carcinogenesis it may be worthwhile to retest this hypothesis under more rigorous conditions by fractionating a higher dose delivered to only a small volume of breast tissue.

An in vitro irradiation experiment has been started. One block of breast tissue from the inguinal area was removed, exposed to 800 R of x irradiation in vitro and returned to the dorsum of the same animal while the contralateral area was autotransplanted without irradiation. Interim results consist of a few mammary neoplasms found only in the irradiated tissue.

Because the Lewis strain of rat is an inbred strain and this offers certain advantages in transplantation experiments, a small group of Lewis rats is being compared to Sprague-Dawley rats in terms of mammary neoplastic response to total body irradiation. Interim results indicate the Lewis rats give a considerably smaller yield of mammary neoplasms than do Sprague-Dawley rats.

Since the experiments of Vogel, that are incomplete, have indicated an RBE of approximately 40 for fission neutrons in producing mammary neoplasia, a group of rats were exposed to a graded series of doses at the Medical Research Reactor in an effort to gather additional data on the RBE of fission neutrons for mammary neoplasia induction. (Shellabarger)

17. Expected Results in FY 1971:

A more precise definition of the incidence of the lens opacities in man as a function of age will be attempted. The latter data will not be combined with that of the unexposed Marshallese, however, until it is evident that there is no basic difference between the two geographical groups. These unexposed data, showing normal incidence of minute opacities with age will constitute a baseline, against which persons exposed to radiation of known physical characteristics may be meaningfully compared. Persons in this category include those exposed in 1958 at the Vinca reactor excursion and in the ORNL Y-12 criticality accident. A comparison of lens findings between these two groups might be pertinent in view of the extensive discussions in the literature of their relative radiation exposures and hematological findings.

17. Expected Results in FY 1971: (Cont'd.

Studies on radio-induced lens opacification will involve: 1) exposures to fast neutrons of previously untested energies, 2) hyperbaric exposures in 100% oxygen and hypoxic exposures to fast neutrons of previously tested energies, and 3) a revised experiment dealing with x-ray fractionation. Histological investigations to parallel the recent radiobiological experiments will include an attempt to determine the actual numbers of germinal cells in the lens epithelium, utilizing tritiated thymidine, and study of the hyperplastic anterior polar opacity. This easily-located unifocal lesion differs markedly in appearance, development and response to radiation from the multifocal posterior subcapsular opacities. (Bateman)

Mature Drosophila sperm will be irradiated with a mixed dose of neutrons and x rays in an attempt to detect interaction between chromosome lesions produced by x rays and similar lesions produced by neutrons. If such an interaction can be detected, the rejoining distance between chromosome lesions will be calculated. At present only upper limits of this distance can be estimated. The finding of such an interaction will also eliminate one of two models that explain observed linear dose-response curves for neutron-induced chromosome exchange and translocations. Recently, Savage has proposed a new hypothesis for chromosome exchange that differs fundamentally from the generally accepted hypothesis of Wolff. Mature Drosophila sperm of various karyotypes will be irradiated and their translocation frequencies determined to test these two hypotheses. (Gonzalez)

Studies of radiation effect on the elasmobranch brain will be continued. Animals exposed earlier will be sacrificed and examined for late changes related to their radiation exposure. Additional animals will be irradiated and put in holding tanks for late effects studies.

A second series of monkeys will be operated upon using the split brain technique and then receive either unilateral or bilateral x-ray exposure of the visual cortex. Since it is too early to define what changes were seen in the animals started last year, the handling of this new group of animals cannot be predicted at this time. For determination of decrement in visual acuity, two additional groups of monkeys will be exposed with doses in the 500 - 1,500 rad range and tested in the same manner as the animals previously exposed at 3,500 rads. Depending upon the outcome of this study additional animals will be exposed and tested to determine the threshold for the radiation effect in terms of decrement in visual acuity. (Carsten)

In the studies on radiation carcinogenesis two new experiments are planned. First, the mammary neoplastic response to a dose-rate of 0.035 R/min. will be compared to a dose-rate of 50 R/min. at various doses. Secondly, the split-dose technique will be applied with partial body irradiation procedures in an effort to learn if repair processes

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Early and Late Effects of Radiation of Different Quality and at Different Dose Rates 06-01-01-(a)

17. Expected Results in FY 1971 (Cont'd.)

are important in radiation carcinogenesis. Experiments on the RBE study of fission neutrons, the effect of age at time of exposure, the Lewis rat sensitivity study, and the in vitro irradiation study should be completed. (Shellabarger)

18. Expected Results in FY 1972:

It is expected that lens opacification studies will be discontinued with the departure of the principal investigator. It may be possible to continue some studies on a collaborative basis. (Bateman)

Drosophila studies will continue based on findings during the previous year; experiments involving Stage 7 and Stage 14 oocytes will be initiated. (Gonzalez)

Continuation of experiments on the central nervous system is planned but is dependent on financial support of collaborating organizations. (Carsten)

Additional carcinogenesis studies are planned but the exact experimental design will depend upon the results of experiments completed in the interim. Extensive studies on the RBE of 0.43 and 1.4 MeV neutrons for rat mammary neoplasia are planned in this year, or sooner if possible, depending upon animal care space and the availability of neutrons. In vitro experiments will be extended in an effort to study the initial events in radiation carcinogenesis in more detail. Also, interactions between high LET radiation and chemical carcinogens on rat mammary carcinogenesis will be studied.

If it is possible to employ a senior scientist of the caliber of L. Feinendegen, a new program can be pursued in depth, in which both the short-term and long-term effects of the incorporation of H-3, I-125, and I-131 into nucleic acids will be studied. A portion of this project will focus upon the toxicity of H-3 and the effect of the position of H-3 in rings upon its biological effect. In connection with these studies, radiation effects will be compared when produced by either H-3 or I-131 incorporation into DNA. Finally, I-125 incorporated into DNA will be studied and compared to I-131 in regard to radiation effects of the two radionuclides. Additional emphasis will be placed upon studying the effects of I-125 because this radionuclide emits Auger electrons. Thus, the disintegration of an I-125 atom offers additional possibilities for producing biological change over and above the conventional release of radiant energy. The effects of H-3, I-125 and I-131 incorporation into nucleic acids will be studied from the standpoints of biochemistry, cell kinetics, and carcinogenesis. (Shellabarger)

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Early and Late Effects of Radiation of Different Quality and at Different Dose Rates 06-01-01-(a)

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

No unusual equipment is required for the various research studies. It is expected however, that further work on modification of the Van de Graaff generator and on the target areas will require approximately $86,000 capital funds in FY 1971 and $40,000 in FY 1972.

20. Proposed Obligations for Related Construction Projects, if any:

Bio-Med conjunct facility.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory
Laboratory

06-Biology & Medicine
Program

1. Contractor:
Associated Universities, Inc.

Contract No.: AT-30-2-GEN-16

Task No.:

2. Project Title:

Effects of Radiation on Living Organisms - Medical Research Mechanisms and Radiosensitivity of Immune Reactions

189 No.: 06-17

3. Budget Activity No.:

06-01-01-(b)

4. Date Prepared:

May 1970

5. Method of Reporting:

BNL Annual Report
BNL Monthly Letter to AEC
Bulletin of the Medical Department

6. Working Location:
Brookhaven National Laboratory

PRIVACY ACT MATERIAL REMOVED

7. Person in Charge:

R. D. Stoner
L. D. Hamilton

Principal Investigator:

R. D. Stoner
L. D. Hamilton

8. Project Term:

Continuing

From: To:

9. Man-Years:

Direct Man-Years	FY 1970	FY 1971	FY 1972
Scientific & Professional	3.4	1.0	2.0
Other	10.1	8.0	10.1
Guests & Res. Collaborators	-	1.0	1.0
Total	13.5	10.0	13.1

10. Costs (In Thousands of Dollars):

	FY 1970	FY 1971	FY 1972
Research Division	251	165	214
Hospital Division	24	15	46
Research Costs	275	180	260

11. Reactor Concept:

12. Materials:

PRIVACY ACT MATERIAL REMOVED

Effects of Radiation on Living Organisms - Medical Research
Project Title: Mechanisms and Radiosensitivity of Immune Reactions 06-01-01-(b)

13. Publications

Savel, H., Kim, C. W. and Hamilton, L. D. Synthesis of radioactive trichinella spiralis larval antigen in vitro. Exp. Parisitol 24, No. 2, 171-5 (1969).

Laissue, J., Hess, M. W., Stoner, R. D., Riedwyl, H. and Cottier, H. Regional disparity of germinal center development in neonatally thymectomized mice after stimulation with tetanus toxoid. Lymphatic Tissue and Germinal Centers in Immune Response, Siore-Donati and A. Hanne, Editors, pp. 285-92, Plenum Press, New York, 1969.

Sordat, B., Sordat, M., Hess, M. W., Stoner, R. D. and Cottier, H. Specific antibody within lymphoid germinal center cells of mice following primary immunization with horse-radish peroxidase: a light and electron microscopic study. J. Exp. Med. 131, No. 1, 77-91 (1970).

Stoner, R. D., Straub, R. F., Moore, W. H. and Jesseph, J. E. Radiosensitivity of immune responses to 2.2 BeV protons γ-radiation and x-radiation. Radiation Effects 2, 97-104 (1969)

14. Scope:

The study of immune mechanisms in one of the major programs in this budget activity is comprised of three interdependent areas of research: (1) the repressive effects of ionizing radiations on antibody responses, (2) radiation-enhanced susceptibility to anaphylactic shock, and (3) proliferation of lymphoid germinal centers in lymphoreticular tissues after antigenic stimulation. Emphasis is directed in these studies to the highly immunogenic properties of complexes of antigen and antibody in eliciting earlier and enhanced antibody responses, as compared with the same antigen (tetanus toxoid) administered either in soluble or adsorbed form. In order to provide a broad basis for the efficacy of primary immunization with complexed antigens, two additional and unrelated antigens, bovine serum albumin (BSA) and horseradish peroxidase (HRP) are used in comparative experiments. The work with BSA is in collaboration with Terres (Tufts University). The studies with tetanus toxoid and HRP are carried on with Cottier, Hess, Sordat, and Laissue (University of Bern, Switzerland) and Odartchenko (Cancer Institute, Lausanne, Switzerland).

The protection afforded by normal immune responses in the prevention and control of infectious diseases may be severely repressed and/or abolished by whole-body exposure to sub-lethal doses of radiation. On the other hand, normal immune responses need to be repressed for successful transplantation of tissues and organs. Antibody responses in normal individuals may also give rise to serious problems, such as hay fever and immediate anaphylactic shock. Previous work in this Laboratory has demonstrated an increased susceptibility of irradiated mice to fatal anaphylactic

14. Scope: (Cont'd.)

shock. Since enhanced antibody responses may be obtained in normal and irradiated mice with antigen-antibody complexes, studies are designed to explore the anaphylactogenic properties of complexes of antigen and antibody formed in antigen excess, equivalence and antibody excess.

Present findings show a close association between the appearance and growth of germinal centers in popliteal nodes and the appearance of serum antibody. Immune defects in so-called immunologic deficiency syndromes in man may result from developmental disorders of immunologically active tissues. Developmental failure of a lymphoid tissue may result in a functional deficiency, absence of one or more immunoglobulins and in morphologically detectable defects. It seems likely that new germinal centers may have their origin from sensitized cells, i.e., cells previously exposed to a specific antigen. Although germinal centers contain immunoglobulins as shown by immunofluorescent methods, it is apparent that most immunoglobulins lie on the surface of cells or in reticular cells within germinal centers. Present findings with HRP antigen provide evidence for antibody formation by lymphoid germinal center cells as well as persistence of antigen and/or HRP-antibody complexes between dendritic reticular cells. Increasing emphasis will be given to primary immunization with antigen-antibody complexes prepared with IgG globulins. Complexes of tetanus toxoid and specific human IgG are also being prepared for primary immunization in man. The long-range purpose of this research is to extend the principles of enhanced immunization demonstrated with the present complexed antigens to situations where antigens of bacterial, viral, protozoan and parasitic origin are poorly antigenic. (Stoner)

In the other major program in this activity the mechanisms of delayed hypersensitivity and antibody production continue as long-range objectives. Short-term objectives include: (1) the effect of immune serum in the transfer of delayed hypersensitivity with cells from animals repeatedly sensitized to x-irradiated recipients, as well as the antibody response in the x-irradiated recipients; (2) the effect of repeated sensitizations on the transformation of sensitized lymphocytes in vitro; (3) the ability of various purified extracts of the T. spiralis antigen to induce and transfer delayed hypersensitivity and/or antibody production; and (4) the effect of synthetic double-stranded RNA on delayed hypersensitivity and/or antibody production.

The aim is to master the immune response for the betterment of the treatment of man. As clinical staff is available studies will be resumed on diseases in which the etiology appears to be the production of antibodies against the individual's own tissues--auto-immune diseases--such as rheumatoid arthritis and demyelinating diseases of the nervous system, e.g. multiple sclerosis. Those diseases, e.g. Hodgkin's disease, in which there is immunological impairment as a result of the replacement of normal immunological tissue by malignant tumor also will be studied. The possibility that Hodgkin's disease may arise as a result of somatic

14. Scope: (Cont'd.)

mutation induced by hyperstimulation of the immune system is of interest. There is a probability that many so-called auto-immune diseases may be due to environmental causes, including radiation, as a result of induction of somatic mutation in the immune tissue, or by the chance complexing of chemicals from the environment with the body's own cells or by the physical agent altering the cells' membranes so that they are recognized as foreign. Such complexes or altered cells then act as antigens, giving rise to antibodies against the tissue previously complexed or altered. Studies on the immune mechanisms in these conditions are designed to understand these processes, to block or alter some of the reactions for therapeutic purposes. Clinical studies, however, are dependent upon the necessary clinical staff.
(Hamilton)

15. Relationship to Other Projects:

Related works include studies by E. P. Cronkite et al on homograft rejection and R. A. Conard on lymphocyte cultures at BNL; W. Taliaferro et al, Argonne National Laboratory, on radiosensitivity of antibody responses; N. Gengozian, Oak Ridge, on bone marrow transplantation immunity; O. Vos, TNO Radiological Defense Lab., The Netherlands, on bone marrow transplantation; D. van Bekkum et al, Radiological Institute, TNO, The Netherlands, on immunology of transplantation; H. Micklem, University of Edinburgh, Scotland, on radiation and transplantation; E. Betz and L. Simar, University of Liege, Belgium, on radiation and antibody production; M. Simic et al, Boris Kidric' Institute of Nuclear Sciences, Yugoslavia, on radiation and antibody production; M. Silverman, U. S. Naval Biological Laboratory, Oakland, on cellular and antibody responses in transplantation; and F. Fitch and R. Wissler, University of Chicago, on radiation and antibody formation.

Although many of the above investigators work on closely allied immunological problems, studies here differ from their projects in three basic conditions: (1) the major antigen used (tetanus toxoid) is commonly used in man; (2) a functional antigen-antibody test system (neutralization of potent tetanus toxin) is used to measure antibody titers; and (3) the immunogenic properties of complexes of tetanus toxoid and specific IgG globulins are being tested in normal and irradiated mice. (Stoner)

Other related studies include work on the lymphocyte by Makinodan at Oak Ridge National Laboratory; on the fate and function of the lymphocyte, especially in the homograft reaction and the graft vs. host reaction, by Gowans at Oxford, and by Medawar and Mitchison at the National Institute for Medical Research, London.

The role of lymphocytes in the transfer of delayed hypersensitivity on the homograft reaction is being studied in man by Lawrence at New York University; in guinea pigs by Chase at Rockefeller Institute and Bloom at Albert Einstein. Benacerraf at the National Institutes of Health and Dixon and associates at La Jolla are investigating the role of leukocytes

(See Continuation Sheet)

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

in the transfer of immune reactions. Many laboratories are studying the effects of phytohemagglutinin (PHA). (Hamilton)

16. Technical Progress in FY 1970:

Histological evaluation of cellular proliferation and de novo formation of germinal centers in regional popliteal nodes was completed. Mice were given a primary immunization in each hind leg foot pad of either fluid tetanus toxoid (FTT) or FTT in complex at equivalence with mouse tetanus antitoxin. Preliminary results indicate that the complexed antigen elicits earlier proliferation as well as more new germinal centers than the same amount of FTT injected alone. Additional findings (Cottier et al) provided new evidence for antibody formation by lymphoid germinal center cells of regional lymph nodes of mice immunized with horseradish peroxidase (HRP). The persistance of HRP antigen and HRP-antibody complexes between dendritic reticular cells was also observed.

A series of experiments on comparative radiosensitivity of primary and secondary antibody responses elicited with the same amount of FTT in complex with different amounts of antibody indicate the following: (1) complexes formed in antigen excess or at equivalence are more effective than complexes in antibody excess in eliciting both primary and secondary antitoxin responses in mice; (2) FTT given alone as a booster injection is more effective than the same amount of FTT given in a complex (in vivo complexing occurs in this situation, when animals have experienced an earlier primary immunization); and (3) irradiated mice (400-500 rads) recover the capacity for responding to antigenic stimulation earlier (8-10 days) when the toxoid is injected in a complex in antigen excess or at equivalence.

In previous studies here, it was reported that there were very high antibody responses to BSA in normal and irradiated mice (300 rads) when the animals were primed with a complex formed at equivalence of BSA and rabbit anti-BSA. A second injection of BSA given 3 to 6 days later elicited high antibody responses within 10 days. A series of similar experiments were carried out in normal and irradiated mice (400 rads) primed with a complex of FTT and mouse antitoxin formed at equivalence. Radiation was delivered one hour before a second injection of FTT on days 3, 6, 9, and 12. Enhanced antitoxin responses were obtained in both normal and irradiated animals. In general, the irradiated mice produced higher titers of antitoxin than corresponding control animals. A tenable explanation for this phenomenon is not evident at this time. (Stoner)

Studies on delayed hypersensitivity and its transfer by cells from sensitized animals were continued. When donor animals were sensitized 4X (1.5 mg total protein) at 2-week intervals and skin-tested, reactions began as small, slightly hemorrhagic spots very early (within 1 hr), reaching their peak between 6-12 hr and decreasing in size and intensity

(See Continuation Sheet)

16. Technical Progress in FY 1970:(Cont'd.)

thereafter. At its peak, the reaction was diffused, slightly hemorrhagic, circumscribed by pale edema, and significantly smaller in size than the typical delayed response.

Antibody was detected by passive cutaneous anaphylaxis (PCA) in all donors even when serum samples were diluted to 1:320. Precipitating antibody was detected by the ring test in all donors with titers up to 1:3200. Active systemic anaphylaxis was used to determine whether the circulating antibody present in donors was of the quality and quantity to cause death when challenged with antigen. Between 22-24 hr after skin test, each repeatedly sensitized donor was challenged intravenously with a shocking dose of antigen (1.90 mg total protein). Approximately 50 percent of donors succumbed as a result of active systemic anaphylaxis. The double-layer immunofluorescent technique showed far more cells from animals repeatedly sensitized fluorescing and more brilliantly than those from animals sensitized only once, implying greater antibody content. Also, a greater number of plasma cells were observed in preparations from repeatedly sensitized donors than from donors sensitized only once.

Stretch preparations of the loose connective tissue of donors, showed typical perivascular infiltration with a predominance (94 percent) of mononuclear cells identical to that seen in donors exhibiting typical delayed hypersensitivity. Under the electron microscope, the lymphocytes were distinct in many ways. The cells appeared less spherical with a more eccentric nucleus and well-developed endoplasmic reticulum filled with electron-opaque material; thus they more nearly resembled antibody-producing plasma cell.

When lymph node cells from repeatedly sensitized donor animals were transferred to normal recipients, skin reactions in recipients were negligible, somewhat like the Arthus rather than the delayed hypersensitivity type. They were negative in many animals. When present, they began as very small, hemorrhagic spots, later circumscribed by pale edema, but the size of the reactions was always negligible.

In x-irradiated (150-200R) recipients, skin reactions were also minimal, whether the donors had been sensitized once or repeatedly sensitized. The only difference was the time at which the reaction reached its peak, i.e., the peak was sooner (between 4-12 hr) in x-irradiated animals receiving cells from donors repeatedly sensitized, and later (between 21-24 hr) in x-irradiated animals receiving cells from donors sensitized only once. Antibody was detected by PCA on day 1 in animals receiving cells from donors repeatedly sensitized. Antibody response in x-irradiated recipients is now being tested.

In studies on transformation of sensitized lymphocytes, in vitro, smears made of lymph node cells from animals sensitized once showed a predominance of small lymphocytes; plasma cells and eosinophils were

(See Continuation Sheet)

16. Technical Progress in FY 1976: (cont'd.)

very scarce. In contrast, in animals repeatedly sensitized, there were many more medium-sized lymphocytes, and a few more large lymphocytes, plasma cells, and eosinophils.

When 1 x 10^5 lymph node cells (almost exclusively lymphocytes) from animals sensitized only once were incubated with 0.1-0.5 ml of antigen for 5 days at 37°, 10-13 percent of lymphocytes from skin-tested animals were transformed into "blast-like" cells; and 6-11 percent of the cells from sensitized but non-skin-tested animals were transformed. When 1 x 10^5 cells from animals repeatedly sensitized were incubated with 0.1-0.5 ml of antigen, 6-11 percent of the cells from skin-tested animals were transformed into "blast-like" cells; and 6-12 percent of the cells from sensitized but non-skin-tested animals were transformed. Approximately 1-2 percent of the cells spontaneously transformed into "blast-like" cells. Thus, repeated sensitizations stimulate antibody production but do not appear to increase the number of lymphocytes committed to transform into "blast-like" cells in vitro.

Experiments using various purified extracts of T. spiralis larvae progressed. Results, thus far, show that skin test reactions are smaller with purified antigens than with the crude saline extract although the peak reaction is at 21-24 hr as expected. The skin test reactions in the recipients were smaller in size and less intense than even in the donors and the reactions did not appear until 22 hr after the skin test. The peak reaction measured 6 x 6 mm in 3 of the animals between 22 and 24 hr. One reason for the minimal reactions with the purified antigens may be due to the fact that these extracts are more dilute than the crude saline extract. The dialyzed antigen proved to be slightly more sensitive in serological test than that precipitated with NH_4SO_4 (80 percent) and then dialyzed. However, both purified extracts were less sensitive than crude saline extract.

Noninfectious double-stranded RNA (Poly rI:rC and Poly rA:rU) was used to determine the effect of delayed hypersensitivity and antibody production to T. spiralis. Up to 300 µg of Poly rI:rC given either before or after sensitization with T. spiralis has no effect on the delayed skin reaction and does not appear to accelerate the antibody response as detected by PCA and precipitin test. When the polynucleotide is given to repeatedly sensitized animals, at the time of the 4th sensitization, antibody is detected by the ring test 1 day after the 4th sensitization and polynucleotide injection. However, there appears to be some interference to the PCA antibody so that a positive reaction is not observed. More interestingly, some of the animals die from anaphylactic shock within minutes after injection of the shocking dose of antigen. Therefore, either the polynucleotide is acting as a spreading factor so that the intradermally injected serum does not remain localized but is spread systemically, or it is binding the 7S, 1 antibody (presumably PCA

(See Continuation Sheet)

16. Technical Progress in FY 1970: (Cont'd.)

antibody is IgG so that it is inactivated and thus made unavailable to react with the shocking dose of antigen.

To date 96 studies have been completed (57 additional studies since last report) on the response of human lymphocytes in vitro to phytohemagglutinin (PHA) measured quantitatively by noting the increase in DNA synthesis from the uptake of tritiated thymidine and by the transformation of small lymphocytes into large, blast-like pyroninophilic cells after 3-5 days of incubation with PHA. These experiments have been carried out on the lymphocytes of 14 normal subjects (7 additional since last report) and 26 patients (19 additional since last report) having unequivocal evidence of multiple sclerosis.

In addition to the effect of PHA of lymphocytes from normal subjects and multiple sclerosis patients, the response of these lymphocytes to the serum and spinal fluids of patients with multiple sclerosis and other neurological problems has been followed. These data are now being programmed for computer analysis. Preliminary impressions of the results are that the spinal fluids but not the serum from patients with multiple sclerosis contain substances that stimulate the PHA-induced transformation of lymphocytes from normal subjects and from multiple sclerosis patients. (Hamilton)

17. Expected Results in FY 1971:

It is planned to complete an experiment of long duration concerning cellular kinetics of lymphoid tissues in mice during secondary tetanus antitoxin responses. The booster injection of toxoid was given 4 days prior to an acute exposure of 600 rads. Tritiated histidine was given at various times to determine the time of incorporation of tritium into antibody in both normal and irradiated animals. These results will be compared with a histological evaluation of the lymphoreticular tissues.

A series of experiments will be carried on to determine the anaphylactogenic properties of complexes of tetanus toxoid and mouse antitoxin. The capacity to elicit anaphylactic shock will be tested in normal and 7-day post-irradiated mice. Complexes will also be prepared with IgG isolated from mouse antitoxin.

Preliminary findings indicate ability to obtain precipitating antibody as well as combining antibody in mice against horseradish peroxidase (HRP). It is planned to characterize the properties of the anti-HRP so as to prepare complexes for Cottier et al to use in study of the localization of complexes in germinal centers.

Complexes of tetanus toxoid and specific human IgG will be prepared to test the efficacy of primary immunization with complexed toxoid in man. The study will be done in collaboration with Dr. C. Jansen (Pretoria).
(Stoner)

(See Continuation Sheet)

17. Expected Results in FY 1971: (Cont'd)

Fine structural studies of lymphocytes from repeatedly sensitized donors should be completed and with documentation in more detail of the sequence in the gradual change of a lymphocyte into a plasma-like cell after stimulation by a specific antigen. It is expected that minimum progress will be made in studies related to the mechanism of delayed hypersensitivity due to reduction of staff. It is hoped however, to continue studies on the transfer of delayed hypersensitivity and antibody production in x-irradiated recipients to determine differences and/or similarities in their mechanisms. Further studies of purified extracts of T. spiralis may separate antigenic fractions responsible for delayed hypersensitivity and those responsible for antibody production. The study by immunofluorescence of antibody content of transformed cells should be continued to pinpoint the cells responsible for the delayed hypersensitivity response. If possible, the effect of synthetic polynucleotide complexes on the delayed hypersensitivity and antibody responses will be studied further as well as the effects of the polynucleotide complexes on interferon with an immunological response to Trichinella.

Manuscripts will be prepared on the response of lymphocytes from normal subjects and patients with multiple sclerosis to PHA, serum and spinal fluid from patients with multiple sclerosis and other neurological disorders, and on the clinical response of patients with multiple sclerosis to short-term treatment with ACTH. (Hamilton)

18. Expected Results in FY 1972: (Cont'd)

Since exposure to ionizing radiations renders mice very susceptible to anaphylaxis, an effort will be made to determine the mechanism altered in this situation. The uterine horns from immunized and irradiated mice will be used as an *in vitro* test system in initial experiments.

The study of the immunogenicity of antigen-antibody complexes will be extended to antigens obtained from parasitic, protozoan, viral and bacterial organisms. Every effort will be made to provide a test challenge for the active immunity induced by immunization with complexed antigen. (Stoner)

Investigations will continue to be designed to pinpoint the cells responsible for the transfer of the delayed hypersensitivity response and how they accomplish this and the relationship of these cells to antibody production. However, in view of the curtailment of professional staff in this area the studies will be reduced markedly in scope. (Hamilton)

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

None

20. Proposed Obligations for Related Construction Projects, if any:

Bio-Medicine Facility

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory
Laboratory

06-Biology & Medicine
Program

1. Contractor:
Associated Universities, Inc.

Contract No.: AT-30-2-GEN-16

Task No.:

2. Project Title:

Molecular & Cellular Radiobiology--
Medical Research

189 No.:
06-57

3. Budget Activity No.:
06-01-02

4. Date Prepared:
May 1970

5. Method of Reporting:
See sub-activities

6. Working Location:
Brookhaven National Laboratory

PRIVACY ACT MATERIAL REMOVED

7. Person in Charge:
See sub-activites

Principal Investigator:

See sub-activities

8. Project Term:
Continuing

From: To:

9. Man-Years:	FY 1970	FY 1971	FY 1972
Sci., Res. Assoc. (Ph.D. or Equiv.)	6.0	6.0	6.0
Visiting Sci.	-	-	-
Prof. (B.S. or Equiv.)	2.5	3.0	3.0
Sci. & Eng. - Total	8.5	9.0	9.0
Technical	8.5	12.5	13.5
Adm. & Service	4.5	4.5	5.0
Guests & Research Collaborators	8.0	6.0	9.5
Total	29.5	32.0	37.0

10. Costs (In Thousands of Dollars):	FY 1970	FY 1971	FY 1972
Labor (including benefits)	346	380	414
Mats., Trav., Dev. Subcont., Spec l Proc.	59	47	63
Reactor, Accel., and/or Computer Usage	3	3	6
Allocated Technical Services	17	14	15
Gen. & Adm. Overhead			
Total Research Cost			

PRIVACY ACT MATERIAL REMOVED

11. Reactor Concept:

12. Materials:

SUMMARY

Sub-Activity	Title
	Medical Research
06-01-02-(a)	Chemistry and Function of Labeled Proteins and Amino Acids
06-01-02-(b)	Granulopoiesis. Studies on the Origin, Rate of Proliferation, Function and Fate of Cells in the Intact Mammal
06-01-02-(c)	Mechanisms of Action of Free Radicals and Hormones

(See Continuation Sheet)

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory — Laboratory

06-Biology & Medicine — Program

PRIVACY ACT MATERIAL REMOVED

1. Contractor: Associated Universities, Inc.
Contract No.: AT-30-2-GEN-16
Task No.:

2. Project Title:
Molecular and Cellular Radiobiology - Medical Research
Chemistry and Function of Labeled Proteins and Amino Acids
189 No.: 06-59

3. Budget Activity No.: 06-01-02-(a)

4. Date Prepared: May 1970

5. Method of Reporting:
BNL Annual Report
BNL Monthly Letter to AEC
Bulletin of the Medical Department

6. Working Location:
Brookhaven National Laboratory

7. Person in Charge:
E. A. Popenoe
D. D. Van Slyke

Principal Investigator:
E. A. Popenoe
D. D. Van Slyke
R. B. Aronson

8. Project Term:
Continuing
From: To:

9. Man-Years:

Direct Man-Years	FY 1970	FY 1971	FY 1972
Scientific & Professional	3.0	3.0	3.0
Other	2.7	2.5	1.6
Guests & Res. Collaborators	1.0	1.0	1.5
Total	6.7	6.5	6.1

10. Costs (In Thousands of Dollars):

	FY 1970	FY 1971	FY 1972
Research Division	135	130	150
Hospital Division	0	0	0
Research Costs	135	130	150

11. Reactor Concept

12. Materials:

PRIVACY ACT MATERIAL REMOVED

1178758

Project Title:

13. Publications:

Popenoe, E. A., Aronson, R. B. and Van Slyke, D. D. The sulfhydryl nature of collagen proline hydroxylase. Arch. Biochem. Biophys. 133, No. 2, 486-92 (1969).

Van Slyke, D. D. and LoMonte, A. Monometric determination of nitrate and nitrite. Microchem. J. (in press).

DiFerrante, N. and Popenoe, E. A. Labeling of acid mucopolysaccharides with tritium. II. Purification and fractionation of tritium-labeled heparin. Carbohydrate Res. (in press).

14. Scope:

The objective of these studies is the determination of the biochemical mechanisms of formation and degradation of certain proteins in order to identify their involvement in the etiology of disease and in aging. Collagen is a fibrous protein constituting about one-third of the total mammalian body protein. It is a structural protein, serving as the organic matrix for the body skeleton, and maintains the integrity of tissues and organs. Two amino acids, hydroxyproline and hydroxylysine, are found in mammals only in collagen where they occur in considerable amounts. Hydroxyproline is probably important in assuring the proper folding of the collagen chains--a necessary first step in formation of the fibrils which occur in tissues. Hydroxylysine carries the carbohydrate which is attached to collagen and is also, in part, a participant in intramolecular crosslinks which render the collagen insoluble. These two amino acids are formed from their precursors, proline and lysine, by an enzymatic hydroxylation reaction after these amino acids are incorporated into the collagen precursor protein chain. At the present time the main effort is devoted to analysis of these hydroxylation reactions as a preliminary to study of the role these reactions may play in determining the form in which collagen is laid down in tissues.
(Popenoe, Van Slyke, Aronson)

The fundamental chemical structure of mucins also is under study. Mucins are macromolecules of great viscosity and antigenicity. Alteration of either the rheological or antigenic properties of mucins seems to be intimately involved in the causes or complications of such diseases as cystic fibrosis, chronic bronchitis and pulmonary emphysema. Although the principal goal is to determine the factors contributing to the very high viscosity of cystic fibrosis mucins and how this viscosity might be reduced, studies are carried out on porcine submaxillary mucin because it is readily available and can be purified by well established procedures.
(Popenoe and M. de Salegui, Mt. Sinai School of Medicine-NIH Grant)

Methods of labeling mucopolysaccharides with tritium are also of interest because of the need for labeled products in the study of the metabolism of mucopolysaccharides.
(Popenoe and N. DiFerrante, Baylor U. School of Medicine)

(See Continuation Sheet)

15. Relationship to Other Projects:

Related studies are carried on by: S. Udenfriend, Roche Institute of Molecular Biology, Nutley, New Jersey, on collagen proline hydroxylase from embryonic rat skin; D. J. Prockop, University of Pennsylvania School of Medicine, on substrate requirements and enzyme substrate interactions for collagen proline hydroxylase; and W. W. Pigman, New York Medical College, on chemical structure of bovine submaxillary mucin. Udenfriend is working on the enzyme from rat skin which is similar but not identical to the one from chick embryo studied here. Prockop is using the chick embryo enzyme but his interest is in the nature of the collagen precursor macromolecule which is actually hydroxylated *in vivo* and its interaction with the enzyme. Neither group has succeeded in isolating the enzyme in a pure state, nor are they known to employ the techniques used here. Neither group seems to be studying hydroxylysine biosynthesis. Dr. Pigman is studying the chemical structure of bovine submaxillary mucin which is chemically different from the porcine mucin (the latter more closely resembles human mucins). He is not known to be studying aggregation-disaggregation phenomena of subunits.

16. Technical Progress in FY 1970:

The stimulating effect of bovine serum albumin on collagen proline hydroxylase was found to be almost independent of the sulfhydryl content of the albumin, in contrast to previous assumptions. Sulfhydryl compounds, previously reported to destroy enzyme activity at higher concentrations (1mM), were found to stimulate activity at low concentrations (0.1mM) but their effect bears a complicated relation to the concentration of iron in the solution

Repeated attempts to purify collagen proline hydroxylase by electrofocusing (electrophoretic separation according to isoelectric point) met with no success because the enzyme and many other proteins in the mixture became insoluble near their isoelectric points. This approach to the purification was abandoned, at least for the present. Recent preliminary experiments with analytical polyacrylamide disc electrophoresis indicated that the enzymatically active protein could be separated from about seven other components and recovered from the gel. Very encouraging results were subsequently obtained using preparative disc electrophoresis in polyacrylamide gel. The enzyme so purified showed only one component on analytical disc electrophoresis, but the recovery so far is low.

A new approach to enzyme purification--"affinity chromatography" or adsorption of the enzyme by a specific inhibitor bound to an insoluble support--also shows promise. Proline hydroxylase activity can be removed from solution leaving the bulk of the protein behind when a solution is passed through a small column of either poly-L-proline or gelatin bound to an insoluble support, or through a column of 4% cross-linked, formaldehyde-tanned gelatin spheres. As yet, no method has been found for recovering the activity from the column, probably because of the high affinity of the enzyme for these inhibitors.

(See Continuation Sheet)

16. Technical Progress in FY 1970: (Cont'd.)

Work in two areas was deferred in order to concentrate efforts into fewer directions. Lysine hydroxylation was not studied during the past year but will be resumed in the near future. Work on bound hydroxylysine was deferred. (Popenoe, Van Slyke, Aronson)

In a collaborative investigation, the kinetics of the disaggregation of porcine submaxillary mucin by the nonionic detergent Triton-X-100 was studied at Mount Sinai School of Medicine using viscosity measurements. The disaggregation is an extremely slow process. At 45° the viscosity of a mucin solution in Triton falls to about one-fifth of its initial value during the first four days, but continues to fall at a continually slowing rate over a period of weeks. At 0° the drop in viscosity is too slow to measure. Other detergents were tested and found to be unsuitable for the disaggregation. Guanidine HCl does not reduce the viscosity of the mucin in solution. Methods for determining protein chain end groups in the mucin were improved and it was found that no new end groups appeared during disaggregation. Thus the disaggregation cannot be due to slow proteolysis.

At Brookhaven, the most degraded mucin preparation so far obtained was examined by ultracentrifugation. All mucin solutions behave in a very non-ideal manner in the centrifuge. Thus previous molecular weight determinations were in error. Methods of conducting the experiments and analyzing the data to obtain reliable results now seem to have been found. The average molecular weight of the mucin in Triton falls from approximately 2 million initially to 233,000. The product is heterogenous and there is an indication that a part of the material has a molecular weight as low as 40,000. If the Triton is removed from the solution with ether there is an almost instantaneous reaggregation to an average molecular weight of 893,000. No further change could be detected over the next two weeks. (Popenoe and de Salegui)

The method for separating active, labeled heparin from radioactive degradation products by graded NaCl elution from Dowex-1, X-2, previously reported, was examined further by determining molecular weights of various fractions in the ultracentrifuge. The molecular weight increased with increasing biological activity (and decreasing specific radioactivity), as did the ester sulfate content to a lesser extent. A plot of log NaCl concentration needed for elution from the resin against 1/molecular weight gave a straight line, in agreement with a theory never before tested with Dowex resins. Further work on this project was not possible because of lack of funds for collaborative studies. (Popenoe and DiFerrante)

17. Expected Results in FY 1971:

Continuing studies on collagen will be directed along three lines. First will be the purification of collagen proline hydroxylase by preparative disc electrophoresis and by affinity chromatography. Dr. A. Tometsko,

(See Continuation Sheet)

17. Expected Results in FY 1971: (Cont'd.)

University of Rochester, is preparing some synthetic polypeptides with lower affinity for the enzyme for use in the latter efforts. A serious problem in these studies is an unpredictable disappearance of enzyme activity in several steps of the purification. Efforts will be directed to understanding and preventing this phenomenon.

Secondly, lysine hydroxylase, now believed to be a separate enzyme, will be studied. Efforts to bring about the hydroxylation of significant amounts of protein bound lysine in solution have not yet succeeded although the reaction goes well in tissue minces. A systematic study will be carried out to determine at what stage the hydroxylation fails and why, and how the reaction can be made to proceed in solution as does proline hydroxylation. Synthetic polypeptides may also aid this phase of the work, either as potential substrates or as inhibitors.

Thirdly the role of iron in the hydroxylation mechanism will be studied, using electron spin resonance techniques. Valuable information on the reaction mechanism of several enzymes, e.g. 3,4-dihydroxyphenylacetate-2,3-oxygenase, pyrocatechase, and dopamine-β-hydroxylase, has been obtained by this approach, but no reaction as complicated as the one under study here has yet been investigated. This study should not have to await completely pure enzyme. It will be carried out in collaboration with Dr. Berg and his group, so that progress will depend in part on availability of time on his EPR spectrometer. (Popenoe, Van Slyke, Aronson)

As noted above, porcine submaxillary mucin behaves peculiarly in the ultracentrifuge so that it is difficult to know exactly what its molecular size is in its native state, or how homogenous it is. This area will be examined further using conditions of varying ionic strength and examining the mucin before and after enzymatic removal of the sialic acid. The nature and extent of the heterogeneity observed in the degraded mucin will also be examined both in the ultracentrifuge and by chromatography. The subunit structure of a series of preparations from glands taken from single animals will be examined using the Triton X-100 degradation procedure in order to determine what contribution genetic variation makes to the heterogeneity of the pooled material previously examined.
(Popenoe and de Salegui)

If possible, labeling studies will be extended to other mucopolysaccharides, especially dermatan sulfate, because of the interest in this material for the study of Hurler's syndrome. (Popenoe and DiFerrante)

18. Expected Results in FY 1972:

It is expected that the proline hydroxylase will be available in pure form and probably lysine hydroxylase. It is difficult to know how quickly the EPR studies will progress, but it is planned to examine both enzymes in their interactions with substrates and cofactors by this technique. Only two other enzymatic hydroxylations are known to have the same cofactor requirements as the collagen hydroxylases--thymine-7-hydroxylase

(See Continuation Sheet)

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

and γ-butyrobetaine-β-hydroxylase which forms carnitine. Both of these ought to be examined in the same way for the light they might throw on collagen hydroxylation.

It is also planned to study the role of hydroxylysine and the bound carbohydrates in determining the aggregation state and stability of collagen in connective tissue. Collagen from hard as well as soft tissue will be included in this study in hopes of determining whether or not changes in these components contribute to the process of aging or to the problems associated with it. (Popenoe, VanSlyke and Aronson)

The study of the subunit structure of mucins will continue with emphasis on the comparison of normal human mucins with those from cystic fibrosis patients. A study will be made of other proteins with which mucins interact in their native environment. It is proposed to use the mucin-protein complex to isolate the other proteins, probably by density gradient centrifugation. These other proteins will be examined for biological activity, particularly for effects on membrane transport. (Popenoe and di Salequi)

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

Capital equipment needs include a preparative centrifuge, $7,000.

20. Proposed Obligations for Related Construction Projects, if any:

Bio-Med Conjunct Facility.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory
Laboratory

06-Biology & Medicine
Program

1. Contractor: Associated Universities, Inc.
Contract No.: AT-30-2-GEN-16
Task No.:

2. Project Title:
Molecular and Cellular Radiobiology - Medical Research
Granulopoiesis. Studies on the Origin, Rate of Proliferation, Function and Fate of Cells in the Intact Mammal

189 No.: 06-65

3. Budget Activity No.: 06-01-02-(b

4. Date Prepared: May 1970

5. Method of Reporting:
BNL Annual Report
BNL Monthly Letter to the AEC
Bulletin of the Medical Department

6. Working Location:
Brookhaven National Laboratory

7. Person in Charge:
E. P. Cronkite
H. A. Johnson

Principal Investigator:
E. P. Cronkite
V. P. Bond
A. D. Chanana H. A. Johnson

8. Project Term:
Continuing

From: To:

9. Man-Years:

Direct Man-Years	FY 1970	FY 1971	FY 1972
Scientific & Professional	3.4	4.0	4.0
Other	6.4	9.3	11.6
Guests & Res. Collaborators	2.0	2.0	2.0
Total	11.8	15.3	17.6

10. Costs (In Thousands of Dollars):

	FY 1970	FY 1971	FY 1972
Research Division	226	263	275
Hospital Division	44	52	75
Research Costs	270	315	350

11. Reactor Concept:

12. Materials:

Cronkite Christopher

Project Title: Molecular and Cellular Radiobiology-Medical Research
Granulopoiesis. Studies on the Origin, Rate of Proliferation, Function and Fate of Cells in the Intact Mammal 06-01-02-(

13. Publications

Johnson, H. A. Information theory and biology: a critique and re-synthesis. BNL Lecture Series #68, September 20, 1967.

Johnson, H. A. Liver regeneration and the "critical mass" hypothesis. Am. J. Pathol. 5_, No. 1, 1-15 (1969).

Cronkite, E. P. and Vincent, P. C. Granulocytopoiesis. Series Hematologica (in press).

Johnson, H. A. Adaptive growth without mitosis. Volume on Cardiac Hypertrophy, N. Alpert, Editor, Academic Press, (in press).

Johnson, H. A. Dose to the cell nucleus from tritiated thymidine. Presented at the Oak Ridge AUI Symposium in Medicine No. 12 "Medical Radionuclides: Radiation Dose and Effects", Oak Ridge, Tennessee, December 8-11, 1969.

Cronkite, E. P. and Vincent, P. C. Granulocytopoiesis. Hemopoietic Cell Proliferation, F. Stohlman, Editor, Grune and Stratton, (in press).

Johnson, H. A. Information theory in biology after 18 years. Science, (in press).

Johnson, H. A. and Pavelec, M. A thermodynamic formulation of radiation dose rate. Radiation Res. (in press).

14. Scope:

Characterization of the kinetics of cell proliferation from hemopoietic systems and the factors that control their function, differentiation and proliferation with particular emphasis upon the feedback loops that determine the population size continues to be the major objective in this budget activity.

A provisional model for granulopoiesis has been constructed in which there is a flow of cells from the stem cell through the classical cytologic stages of granulopoiesis. There are single mitoses at the myeloblast and promyelocyte levels and two mitoses at the myelocyte level. The system is believed to be regulated by two feedback loops. The first loop controls the release of mature granulocytes and the second regulates the rate of input of stem cells into granulopoiesis. From the model the following predictions can be made: a) increased myelocyte production can be achieved only by shortening the generation time at the expense of G_1 and this would result in an increased labeling index of myelocytes under stimuli demanding more blood granulocytes; b) there must be a minimum obligatory transit time through metamyelocyte and band compartment--under greatly increased

(See Continuation Sheet)

blood granulocyte turnover rates this would result in a shrinkage in the size of the metamyelocytes, bands and marrow granulocytes, followed by an expansion of the compartments as marrow production rates are increased; c) granulocytes released prematurely under stress should have a longer potential useful life in the blood; d) generalized random loss is curtailed in infection; e) increased marrow production rate at the myelocyte level cannot influence the input of granulocytes into the blood until at least 48 hours after the initiating stimulus; f) demand for granulocytes in excess of certain limits would lead inevitably to neutropenia in response to infection. Major efforts are made to verify or refute these predictions. In addition studies are performed to confirm the possible existence of a cycle in the concentration of neutrophils in the blood.

The possible cyclic nature of granulocytopoiesis has further important implications in clinical medicine. Unless the cycle time and the amplitude of the cycle are known, one could impute toxicity to new drugs on the basis of a granulocyte count at the nadir. On the other hand, observations taken at the peak of the cycle might give a false sense of security. The cycle time and amplitude are of particular importance in detecting effects of radiation in exposed populations or individuals, and studying drug doses of cytotoxic agents in the treatment of metastatic disease where the harmful effects are primarily focussed upon depression of granulocytopoiesis. Attempts are made to perfect a non-fatal method by which the production rate of granulocytes can be increased or decreased at will.

Studies will be designed to see if the "critical mass" concept of control of cell division developed by Johnson to explain renal hypertrophy and hepatic regeneration applies to the control of cell division in hemopoietic tissues and granulopoiesis. (Cronkite)

The relationship between an absolute dose of ionizing radiation and any of its biological effects is entirely empirical. Since such radiation effects are the results of the addition of energy to a living system, it would seem that an absolute dose effect relationship could be expressed in thermodynamic terms. This can be accomplished, to a first approximation, by adding the absorbed radiant energy to the free energy pool of the system with just two constraints: namely, that it is added in an inhomogenous distribution and that excitations are permitted to migrate through a limited distance. This increase in free energy can then be used to predict the occurrence of chemical events in the usual way. A relationship developed along these lines has allowed the prediction of the absolute dose rate required to double spontaneous mutation rates. Where experimental data are available, the theoretical values are in good agreement. (Johnson)

The aim of a completed study was to test the critical mass hypothesis by comparing mitotic indices predicted by the hypothesis. It is generally assumed that organ growth is controlled by regulation of DNA synthesis and

(See Continuation Sheet)

14. Scope (cont.)

cell division. Earlier studies of compensatory growth in the kidney indicated that growth is initiated in the cell cytoplasm, implying that mitosis is merely a secondary consequence of cytoplasmic growth (the critical mass hypothesis). (Johnson)

The transport mechanism for renal tubular reabsorption of protein is not known. Recent electron microscopic studies using hemoglobin or peroxidase have suggested that protein is transported through the cytoplasm of tubular epithelial cells in vesicles which form under the brush border. Thus, the aim of another study is to determine whether this process is the physiological mechanism for reabsorption or whether it simply represents a means of storage and disposal of excess or foreign protein. (Johnson and W. Fingal, Biochiven Semester Program)

Two studies are carried on by an NIH Gerontology Fellow. One is on temperature and length of life in the annual fish, Nothobranchius guentheri. It has been proposed that even mild alteration of an environmental temperature over a prolonged period may significantly affect the life span of poikilothermic organisms. If true, animals reared throughout their entire lives at appropriately different environmental temperatures might help to decide which biochemical parameters of aging are indeed true indicators of this condition. Mortality rates and disease patterns are useful criteria in assessing aging under these conditions. Recently Walford et al described senescent changes in so-called "annual fish", which are reported by aquarists to have a total life span of approximately one year. Life spans were significantly prolonged by retaining the fish at reduced temperatures, and the rate of reduction of soluble/insoluble collagen ratios was slowed substantially. If temperature exerts such an effect upon the rate of aging, then a mild increase in environmental temperature (within the physiological range) should reverse the result, viz. aging should be accelerated. This hypothesis is being tested with N. guentheri.

The other study is on age changes in size and number of mouse hepatic mitochondria in response to partial hepatectomy. Hepatic mitochondria of senile mammalian liver are decreased in number and increased in size without any special qualitative changes in their ultrastructure. Barrows established a relationship with reduced succinic dehydrogenase activity in liver. It has been proposed that in hepatic cells, the mitochondria of the aged are increased in size in order to function more actively in compensation for their number. Further, it has been suggested that such changes are due to incomplete division following synthesis of new mitochondrial material, perhaps due to some inhibitory process. Attempts are made to test this hypothesis by performing partial hepatectomies upon young and senile mice and measuring the regenerative capabilities of their mitochondria. (Johnson and Rotermund)

(See Continuation Sheet)

Project Title: Molecular and Cellular Radiobiology - Medical Research
Hematopoiesis. Studies on the Origin, Rate of Proliferation, Migration and Fate of Cells in the Intact Mammal 06-01-02-(b)

14. Scope: (Cont'd)

Since lowered temperature shortens the life spans of certain cold blooded animals, the question arises as to whether this life shortening also occurs at the cellular level, i.e., whether increasing the temperature shortens the generative cycle of cells. Accordingly, a study was initiated on cell proliferation kinetics of the intestinal epithelium and hemopoietic tissue of goldfish adapted to temperatures of 22°, 30°, 35°, and 38° centigrade. (Johnson and M. Nieto, Spanish Ministry of Industry)

15. Relationship to Other Projects:

Closely related studies are reported in 06-01-01-(b), 06-03-01-(d), and 06-03-01-(e). Studies are being performed on granulocytopoiesis by Fliedner, University of Ulm, Germany; Patt, University of California, San Francisco; Boggs and Chervenick, University of Pittsburgh; and Palmer, University of North Carolina. The studies on the determination of DNA content in myelocytes are performed in cooperation with Dr. Sven Killmann, University of Copenhagen. (Cronkite)

Related studies on the interaction of thermal and radiative energies have been done by Fluke at Duke University and Setlow at Oak Ridge. Related studies on protein vesicle formation, using peroxidase have been done by Graham and Karnovsky at Harvard University. (Johnson)

Walford and Liu, University of California School of Medicine, Los Angeles, initiated work on annual fish. The study on age changes in size and number of mouse hepatic mitochondria in response to partial hepatectomy is an extension of work of Taichi at Nagoya University School of Medicine, Japan. (Johnson and Rotermund)

16. Technical Progress in FY 1970:

Daily granulocyte counts were performed on a series of normal males 26-55 years of age in a study initiated to confirm or refute the existence of a periodic cycle in the concentration of granulocytes. Similar studies were initiated in several hospital patients to see if the amplitude and duration of the cycle varies in disease states and under drug therapy. Studies before and after the administration of L-Dopa in patients with Parkinsonism are of particular interest because of the previously described apparent granulopenia and modest morphological changes in the myelocytes of the marrow. A series of bone marrow aspirations were performed on normal volunteers and the marrow incubated with tritiated thymidine to determine the fraction of cells in DNA synthesis in the various cytological classes. Preparations for determination of DNA content of the various cell classes also were made.

A new non-fatal animal model was designed for the study of granulocytopoiesis and is being utilized. This model embraces the introduction

(See Continuation Sheet)

of turpentine, a treatment that produces sterile inflammation, into the uterine cavity of dogs. At appropriate time intervals the uterus can be surgically removed and the stimulus for granulocytopoiesis turned off. Subsequently the marrow and blood behavior can be observed. To date it has been shown that as soon as the "sink" for granulocytes is removed, peripheral blood granulocyte concentration increases rapidly to a level much higher than in the presence of the inflammatory focus. The granulocyte count falls and finally, in a cyclic manner, is modulated back to the pre-inflammatory level. The influence of several drugs, including L-Dopa, upon the normal periodic cycle of granulocytes in the dog is being ascertained along with the influence of inflammation in order to test whether these and other drugs interfere with the capability of the marrow to respond to life-threatening infectious processes. Experiments to study the effect of low level radiation on the granulocyte cycle of dogs were designed in order to see if a better system to detect radiation effects could be devised.

Studies on the question of ineffective erythropoiesis and granulopoiesis were temporarily postponed because of inadequate technical and professional help. (Cronkite)

The measuring of dose rates of radiation which double the thermal rate of inactivation of dry peroxidase was completed. Observed dose rates are in good agreement with theoretical values. This means that the thermodynamic formulation of radiation dose rate has predictive value.

The plan to test the "critical mass" hypothesis by inhibiting mitosis in the compensating kidney was abandoned for technical reasons; cell sizes were more easily measured in the mouse liver. A theoretical relationship between mitotic index, cell size, and growth rate was derived. Mitotic indices measured at various times after partial hepatectomy were in good agreement with theoretical values. It was shown that the critical mass hypothesis could account for the previously unexplained interplay between cell hypertrophy and cell hyperplasia in the regenerating liver. (Johnson)

In the study of renal tubular reabsorption of protein it was shown that after eight days there is no movement of protein laden vesicles. Evidently this is not the physiological mechanism for protein reabsorption. The finding that intercellular spaces are filled with the peroxidase reaction product within 90 seconds after injection, suggests protein is apparently reabsorbed between epithelial cells of the proximal tubule. (Johnson and Fingal)

Work on the temperature and length of life in the Annual Fish was initiated. The fish have been successfully spawned, are embryos and are presently undergoing maturation, a process taking seven to nine weeks with present hatching techniques. A pilot study using a school of

(See Continuation Sheet)

Molecular and Cellular Radiobiology - Medical Research
Immunopoiesis. Studies on the Origin, Rate of Proliferation,
Project Title: Function and Fate of Cells in the Intact Mammal 06-01-02-(b)

16. Technical Progress in FY 1970: (Cont'd.)

twenty-five inbred mouse that mortality curves are rectangular, with the onset of mortality preceding at a steady rate from seven months. The longest survivors preferably should live one year.

Studies on mouse hepatic mitochondria were begun. A senile population of mice was developed, and many of the techniques of electron microscopy

Project Title: [illegible] Radiobiology - Medical Research
[illegible] on the Origin, Rate of Proliferation, [illegible] and Fate [illegible] Cells in the Intact Mammal 06-01-02-(6)

17. Expected Results in FY 1971 (Cont'd.)

In the collaborative studies under the NIH gerontology fellow program it is hoped that survival curves will establish the effect of temperature on aging rates of N. guentheri; also to supply the data on the nature of mitochondrial biogenesis in young and old mice following hepatectomy.

(Johnson, Rotermund)

Studies of cell proliferation kinetics of goldfish should be completed with accumulation of data on change in rate of DNA synthesis, rate of maturation and length of the generation cycle resulting from increase in ambient temperature (Johnson, Nieto)

18. Expected Results in FY 1972

Studies will be initiated on the nature of the signal controlling the slow feedback loop which regulates the duration of the myelocyte generative cycle and/or recruits stem cells into granulopoiesis. A new concept for the study of normal and malignant hemopoietic cell proliferation is developing that involves the administration of trace amounts of I-125 and I-131 UDR and C-11 thymidine to patients at different time intervals. The movement of cells and areas of proliferation and influence of physiological factors upon them would be measured by utilizing the Brookhaven whole-body counting and computer facility. In addition, the same technology will be used to detect tumor metastases, to measure uptake of metastases or primary tumors before and after test doses of radiation and chemotherapeutic agents in order to select the best agents and time schedules for therapy, and to observe the effect on normal hemopoiesis utilizing Fe-52 and Tc-99m colloid in addition to the labeled pyrimidines

Studies will be commenced on the bio-effect of position of H-3 on pyrimidine rings in mammalian cellular system in vivo and in culture.

To implement these studies will require the service of a scientist of the caliber of Dr. L. E. Feinendegen to replace a terminating scientist, and the addition of two technicians. Also, two research associates will be required in subsequent years.

(Cronkite)

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

Continuous flow blood centrifuge, $60,000. Equipment for ex vivo and in vivo culture systems, $30,000, ($20,000 FY 1971, $10,000 FY 1972).

20. Proposed Obligations for Related Construction Projects, if any:

Bio-Med conjunct facility.

SCHEDULE 159

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory
Laboratory

06-Biology & Medicine
Program

1. Contractor:
Associated Universities, Inc.

Contract No.: AT-30-2-GEN-16

Task No.:

2. Project Title:

Molecular and Cellular Radiobiology - Medical Research Mechanisms of Action of Free Radicals and Hormones

189 No.: 06-73

3. Budget Activity No.:

06-01-02-(c)

4. Date Prepared:

May 1970

5. Method of Reporting:
BNL Annual Report
BNL Monthly Letter to AEC
Bulletin of the Medical Department

6. Working Location:
Brookhaven National Laboratory

7. Person in Charge:
D. C. Borg
I. L. Schwartz (Mt. Sinai School of Medicine)

Principal Investigator:
D. C. Borg
I. L. Schwartz (Mt. Sinai School of Medicine)
R. Walter (Mt. Sinai School of Medicine)

8. Project Term:
Continuing

From: To:

9. Man-Years:

Direct Man Years	FY 1970	FY 1971	FY 1972
Scientific & Professional	2.1	2.0	2.0
Other	3.9	5.2	5.3
Guests & Res. Collaborators	5.0	3.0	6.0
Total	11.0	10.2	13.3

10. Costs (In Thousands of Dollars):

	FY 1970	FY 1971	FY 1972
Research Division	157	152	170
Hospital Division	23	27	25
Research Costs	180	179	195

11. Reactor Concept:

12. Materials:

13. Publications

Fajer, J., [illegible], D. C., Forman, A., Dolphin, D. and Felton, R. H. π-cation radicals and their relation to chlorophyll. J. Am. Chem. Soc. (in press).

Dolphin, D., Felton, R. H., Borg, D. C. and Fajer, J. Isoporphyrins. J. Am. Chem. Soc. (in press).

Walter, R. Further studies with selenium-containing amino acids and peptides. Proc. First American Peptide Symposium, New Haven, Connecticut, August 1965 (in press).

Chiu, C.C., Schwartz, I. L. and Walter, R. Crystal data of a seleno analog of oxytocin. Science 163, 921-2 (1969).

Le Fevre, M. E. Calibration of the Clark oxygen electrode for use in aqueous solutions. J. Appl. Physiol. 26, 844-6 (1969).

Glass, J. D., Schwartz, I. L. and Walter, R. Enzymatic inactivation of peptide hormones possessing a C-terminal amide group. Proc. Nat. Acad. Sci. U.S. (in press).

Eggena, P., Schwartz, I. L. and Walter, R. Osmo-regulation at the periphery: action of hypertonicity on toad bladder permeability. Presented at Symposium on Natriuretic Hormones, Bratislava, Czechoslovakia, August 1969.

Johnson, L. F., Schwartz, I. L. and Walter, R. Oxytocin and neurohypophyseal peptides: spectral assignment and conformational analysis by 220Mc nuclear magnetic resonance. Proc. Nat. Acad. Sci. U.S. 64, 1269-75, (1969).

Roy, J., Gardner, W., Schwartz, I. L. and Walter, R. Optically active selenium-containing amino acids. The synthesis of L-selenocystine and L-selenolanthionine. J. Org. Chem. (in press).

Walter, R., Havran, R. T., Schwartz, I. L. and Johnson, L. F. Interaction of D_2O, Co^{+2}, Ni^{+2} and Cu^{+2} with oxytocin. Presented at the 10th European Peptide Symposium, Abano Terme, Italy, September 1969.

Meienhofer, J., Trzeciak, A., Bajusz, T., Hechter, O., Havran, R. T., Schwartz, I. L. and Walter, R. Solid-phase synthesis and some pharmacological properties of (8-arginine)-vasopressin and (8-arginine) vasopressinoic acid. Presented at the 10th European Peptide Symposium, Abano Terme, Italy, September 1969.

Eggena, P., Schwartz, I. and Walter, R. Action of aldosterone and hypertonicity on toad bladder permeability. Presented at the Symposium on Natriuretic Hormones, Bratislava, Czechoslovakia, August 1969.

(See Continuation Sheet)

13. Publications: (Cont'd.)

Dousa, T., Adler, R. and Schwartz, I. (8-arginine)-vasopressinoic acid: an inhibitor of rabbit kidney adenyl cyclase. Science 167, No. 3921, 1134-5 (1970).

Pande, C. S., Riddick, J. and Walter, R. Synthesis and application in peptide chemistry of amino acids possessing an optically active selenohomocysteine skeleton. J. Org. Chem. (in press).

14. Scope:

Two primary objectives are sought in this budget activity: elucidation of the roles of free radicals as reactive intermediates in some steps of normal and pathological metabolism; and investigation of the hormonal and other mechanisms controlling membrane permeability and transport processes at the cellular and molecular levels. In the former, present efforts are focused on establishing the existence and properties of free radical forms of biologically important substances, such as metalloporphyrins, hormones, nucleic acid and protein constituents, melanin and visual pigments, and some drugs and biochemicals; and seeking to identify the free radicals found in tissues. A broad goal of the program is to use this knowledge for greater understanding and control of some aspects of normal physiology and of disease; but there is scope to extend the work to free radical photochemistry related to environmental contamination. More specific aims have been to correlate radiobiological free radical effects with physiological bioenergetic mechanisms and to test theories relating free radical mechanisms to fundamental natural and radiation-accelerated aging processes.

In the dynamic chemical milieux of living organisms or liquid reaction systems the concentrations of free radicals may be extremely low (perhaps picomolar to nanomolar) even when their properties largely determine the courses of critical reactions. The need to detect relatively small numbers of free radicals in biomedical experimentation has led to the widespread application of electron paramagnetic resonance (EPR) spectrometry. However, EPR requirements regarding the size and other properties of samples are highly restrictive, and the highest sensitivity of current EPR spectrometers may be orders of magnitude too low for much biomedical work. Therefore, instrumental considerations often set the limits to experimentation in this field. Furthermore, the resolution of EPR spectra is intrinsically poor with randomly oriented paramagnets, such as those in tissues or polycrystalline samples; and in other cases EPR spectral complexity defies analysis. Hence, pioneering research groups have been amplifying EPR capabilities with computer data processing and with new double resonance spectroscopies, such as ENDOR and ELDOR.

Studies here investigating bioenergetic free radical mechanisms have utilized EPR to a great extent, with emphasis on special apparatus for maintaining steady states of labile free radicals in liquid reaction systems. Recently computerization, new radical-trapping procedures, ion-irradiation techniques, and special means of examining large samples of frozen tissue have been explored. Application of ELDOR to biomedical and

(See Continuation Sheet) 06-75

Project Title: [illegible] from Radicals and Hormones 06-01-02-(...

14. Scope: [illegible]

Irradiated [illegible] planned, and if ENDOR capability is desired. (Berg)

Within the broad objective to elucidate hormonal and other mechanisms controlling membrane permeability and transport processes at the cellular and molecular levels, studies are concerned with: the correlation of the primary structure of peptide hormones and synthetic analogs with their activity in in vivo and in vitro systems; the elucidation of the intermediary steps between the hormone-receptor recognition and ultimate biological response; and the correlation of the three-dimensional structure of peptide hormones with their physiological properties. (Walter, Schwartz)

15. Relationship to Other Projects:

EPR applications to the life sciences have been increasing rapidly. From the projects and the literature reviewed it is believed that the instrumental developments in the BNL program involve little overlap with work elsewhere, and the major subject areas of BNL's biomedical work on free radical reactions is essentially unduplicated. In addition to the collaborative work reported on below, the following research has relevance: Shields and Hamrick Wake Forest University- EPR and other studies on irradiation defects in single crystals of amino acids and other small biochemicals in the solid state Snipes at Pennsylvania State University--EPR on irradiation defects in single crystals and powders of some amino acids, purines and pyrimidines Fox at Roswell Park Memorial Institute--EPR on irradiation defects, and application of advanced EPR and ENDOR techniques; Pullman at Sloan-Kettering Institute--EPR on irradiated, dry sulhydryl-containing radioprotective agents and on irradiated bone, also studies on free radicals obtained from some carcinogens; Henriksen at Norsk-Hydro's Institute for Cancer Research (Norway)--EPR on irradiated, dry amino acids, peptides, and proteins, including mixtures, effect of radiation quality; Gordy at Duke University- EPR on irradiated crystalline and polycrystalline amino acids and peptides; Manzerall at Rockefeller University--EPR and other studies of oxidation products from porphyrins (This work does overlap studies of porphyrin radicals here, although there is some disagreement in interpretation and thus a little nutrient duplication. There is a real aspect of scientific competition here, however); Sutton at University of Minnesota--EPR of chloroplasts and other photosynthetic units. (This correlates well with the physical chemical experiments here on chlorophyll but does not duplicate the approach); Weaver at NASA/Ames--EPR of photosynthesis in situ; Commoner, et al at Washington University--extensive investigations of EPR signals found in tissues, emphasizes instrumental techniques to study intact tissue rather than analyzing components. (A major program with which attempts here to study large samples of frozen tissues must be correlated); Driscoll at Jefferson Medical College, Pedersen at McGill University, and Emanuel, et al at Academy of Sciences (USSR)--EPR studies of frozen (not pulverized) normal and neoplastic animal tissues; Norman

(See Continuation Sheet)

06-76

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

et al at University of York (England)--use of flow systems for EPR study of transient free radicals in liquid. Other related work includes that of Yamazaki at Hokkaido University, Levy and Mattern, BNL Physics Department, Livingston and Zeldes at ORNL, Smaller at ANL, Calvin, et al at LRL, and Landgraf, et al at Varian Associates (Borg)

Related structural studies on neurohypophyseal hormones are carried on at BNL Biology Department and several university laboratories are involved in related studies on transport processes and membrane properties. (Schwartz, Walter)

16. Technical Progress in FY 1970:

The expected progress for this year in computer applications was exceeded, in that the capabilities for data reduction already are more powerful than those foreseen. This was accomplished by writing an extensive Fortran program (CONVOL) for processing arrays of spectral data using the CDC-6600. Routines were developed for arithmetic treatment of data arrays, for curve-smoothing and filtering using several different methods, for shifting spectra and changing both abscissa and ordinate scale factors, for addition and subtraction of spectra, for obtaining (multiple) integrals or derivatives, and for curve shaping and resolution enhancement (using the convolutional method) with any of a number of analytical shape-functions. Subprograms provide for reading and writing magnetic tape in Sigma-2 format to permit high-volume data transfer, for CalComp graphic output, and for punch-card data input and output. Many of the data-processing procedures proven to be of value by CONVOL were then rewritten in Sigma-2 assembly language to be available on-line under teletypewriter control at the EPR spectrometer console. An extensive program for simulation of isotopic hyperfine components of EPR spectra also was written for the Sigma-2, allowing direct intercomparison of experimental and theoretical spectra and greatly facilitating spectral analysis. By the end of 1969 the BNL on-line capability exceeded those of commercial or other known spectral data-processing systems and the system proved very valuable in experimental work.

Although the CONVOL program and the on-line program were developed primarily for EPR spectral data, they are general in format and can be applied to spectra and to data arrays of many kinds, including NMR, optical, beta or gamma spectra, chromatographic data, etc. In one example, CONVOL resolved a digitized spectrum of overlapping chromatographic peaks.

Experimental apparatus and instrumentation problems required a substantial effort. These were partly a result of "old age" component failures in the spectrometer, a vacuum-tube version procured in 1963. In addition, calibrations for high-resolution spectroscopy using the time-averaging capability of the computer system brought out serious

(See Continuation Sheet)

16. Technical Progress in FY 1977 (cont'd.)

limitations in the ability of the spectrometer to retrace magnetic field scans with sufficient precision. Special replacement of certain parts improved performance, but the capability of older versions of EPR spectrometers to interface with computers in this way remains suboptimal. Arrangements were made for factory installation of an external sweep drive to permit accurate computer control of spectra retracing.

The planned development of large sample cavity procedures to the point where a systematic study of tissue EPR signals could be undertaken was not accomplished, but improved dewar systems were made for the large-sample cylindrical EPR cavity, and a large-sample rectangular cavity obtained. It appears that a successful design has been achieved. Double resonance procedures (ENDOR and ELDOR) to support programs investigating tissue free radical sites and to improve resolution of some complex EPR spectra or those from solid materials were evaluated. It was concluded that the present state of instrument development make application of both procedures desirable for research with free radicals. ELDOR apparatus has been ordered, and the necessary redesign of existing equipment was about 80% accomplished.

A collaborative study of porphyrin free radicals with Fajer (BNL Department of Applied Science), Felton (Georgia Tech), and Dolphin (Harvard University) proved highly productive and received a larger effort than anticipated. This work has significance because biological electron transport involves many heme enzymes, and the role of free radical forms remains moot. Furthermore, the photochemical steps of photosynthesis involve free radical species whose exact nature is not yet known, although it is the subject of many investigations. Studies during the year successfully characterized π radical cations and diamagnetic dications of several synthetic porphyrins and elucidated some of the reactions leading to their formation and decay. Means were found of isolating and stabilizing some of the radical forms so that they could be used as chemical reactants in other reactions, a technique that was then used in work on chlorophyll radicals. A species of porphyrin, isoporphyrins, that had been predicted to exist was found, and one example was characterized. Studies were completed that appear to identify the first free radical of photosynthesis in green plants specifically as a π-cation radical of chlorophyll a.

Plans to provide further resolution of the free-radical spectra of visual pigments and to complete definitive computer-based EPR studies on rhodopsin and intact rod outer segments were only partly fulfilled. In conjunction with Freed and Sack (BNL Chemistry Department), further work was done with the cryogenic liquid systems, and a vacuum line was built to facilitate the EPR work. A phosphatidylethanolamine Schiff's base of trans-retiral was synthesized (recent work indicates 11-cis retinal exists in such a compound in native rhodopsin), but the material was unstable, and the work was inconclusive. However, the low-temperature liquid system was applied with success to the question of whether a true charge-transfer or a photosensitized reaction had been observed previously;

(See Continuation Sheet)

16. Technical Progress in FY 1970: (Cont'd.)

the latter alternative was ruled out indicating the anion-radical nature of the retinal free radicals. An improved method of rhodopsin isolation was adopted and an extraction of rhodopsin and rod cells was achieved with greater yield than before.

In the studies relating radiation and aging a special vacuum line with facilities for hydrogen and/or deuterium gas injection in a region of microwave discharge was fabricated. (The hydrogen or deuterium atoms produced in the discharge attack molecules in the same way as do some of the active species produced by ionizing irradiation, but the reactions are "cleaner" and more easily analyzed, in general.) This system plus gamma-irradiation were used to extend previous EPR studies on irradiation damage to certain purines and pyrimidines in dry powders. In particular, the damage center in 9-methyl adenine was much better described as a result of this work. Single crystals of the substance were successfully grown to aid in completing the study. Simultaneously, the rapid-flow apparatus developed last year for Q-band EPR studies of reactions with three fluid components was proved out and calibrated, and changes were made in the syringe pump in preparation for the extension of the investigation from dry samples to dynamic liquid systems.

EPR examination of melanin-like pigments formed non-enzymically from dopa and dopamine gave evidence of identifiable free radical intermediates, a strong pH effect on the EPR behavior of the product pigments, and some suggestion that at least the synthetic melanins made from dopa and dopamine may be distinguishable by EPR.

Plans to complete the analyses of at least some of the complex radicals of nicotinamide derivatives whose EPR spectra had been recorded previously and to assay new methods of producing pyridine nucleotide radicals within the EPR cavity were handicapped by reduced collaborative support and the fact that the nicotinamide compounds had degraded with time. Nonetheless, it was possible to produce a new free radical by oxidation of N-methyl nicotinamide, and incomplete EPR spectrum analysis indicated absence of the dimerization previously seen with radicals formed by reduction; although final analysis will require a better signal to allow full resolution of the EPR spectrum. (Borg)

The solid phase procedure of peptide synthesis recently developed by Professor Merrifield at Rockefeller University was used to prepare the first neurohypophyseal hormone analog (8-arginine-vasopressinoic acid), which potently inhibits the antidiuretic effect of ADH. A specifically labeled C-terminal gastrin tetrapeptide analog was synthesized, tert-butoxycarbonyl-L-tryptophyl-L-norleucyl-4,5-^{3}H-L-aspartyl-L-phenylalanine amide. This labeled peptide was obtained by catalytic tritiation of tert-butoxycarbonyl-L-tryptophyl-L-4-dehydronorleucyl-L-phenylalanine amide (II). Upon catalytic hydrogenation, the latter compound gave

(See Continuation Sheet)

tert-butoxycarbonyl-L-tryptophyl-L-tyrosyl-L-aspartyl-L-phenylalanine amide (III), which possessed physical properties identical with those of the authentic material. The biological properties of compounds II and III are compared with those of L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine amide, the natural C-terminal tetrapeptide of gastrin, with respect to gastric secretion in the frog, rat, and dog, and pancreatic secretion in the dog.

With the recognition that a selenium compound (Factor 3) exercises a protective effect against certain nutritional deficiencies, the emphasis in selenium research shifted to an effort to elucidate its role as an essential element. This development was paralleled by the search for selenium compounds in the plant and animal kingdoms. In this context it became important to find a method for the separation of organic, selenium-containing compounds from the corresponding sulfur isologs. It was found that racemic Se-methylselenocysteine, selenocystine, selenomethionine, selenoethionine and Se-benzylselenocysteine are separated from their corresponding sulfur isologs by ion-exchange chromatography. The reason for previous failures to obtain reproducible color values for selenocystine was uncovered. The selenium-selenium bond of selenocystine is cleaved by mercaptans and selenols, yielding acyclic molecules with selenium-sulfur or selenium-selenium bonds. The diselenide-sulfhydryl and diselenide-selenol interchange reactions, which occur over a wide pH range, must be taken into account during analytical investigations involving mixtures of diselenides and/or thiols, and/or selenols. Treatment of peptides containing selenocystine or selenocysteine residues (1-seleno-oxytocin, 6-seleno-oxytocin, deamino-1-seleno-oxytocin, deamino-6-seleno-oxytocin and deamino-di-seleno-oxytocin) in 6N HCl at 110° for 22 hours partially degraded selenocystine; however, when selenocystine itself was treated under identical reaction conditions, it was almost entirely destroyed.

A method developed earlier, consisting of a nucleophilic displacement of the O-p-toluensulfonate moiety of an O-tolylated L-serine derivative by the benzyl selenolate anion, is advantageous for the preparation of Se-benzyl-L-selenocysteine compounds which bear selectively removable amino- and carboxyl-protecting groups. In order to demonstrate the wider scope for this displacement reaction in the synthesis of selenium-containing amino acids, the possibility of transforming an O-tolylated L-serine derivative with sodium hydrogen selenide to the corresponding selenocysteine derivative was investigated. Such a derivative with its free selenol function would provide a key intermediate allowing the transformation to either the diselenide by oxidation or to selenides by alkylation. While the former type of reaction would pave the way for a convenient synthesis of L-selenocystine, the latter would offer a route toward the synthesis of dialkylselenides which possess a selenocysteine moiety as the basic skeleton, such as L-selenolanthionine. The latter reaction path would also permit the introduction of selectively

(See Continuation Sheet)

16. Technical Progress in FY 1970: (Cont'd.)

removable selenium-protecting groups. Both of these goals were accomplished by the synthesis of L-selenocystine and L-selenolanthionine.

In the recently described synthesis of several NPS-protected N-hydroxysuccinimide esters of amino acids there is no report of the corresponding glutamine and asparagine derivatives. The preparation of these two derivatives became of interest in the course of peptide synthesis involving the solid phase procedure of Merrifield in order to avoid the possibility of nitrile formation previously observed during peptide elongation with both glutamine and asparagine derivatives when DCCI was used as a coupling reagent. However, in attempts to prepare the N-hydroxysuccinimide ester (II) of NPS-L-glutamine (I) by the mixed anhydride and the DCCI methods, the sole product isolated was NPS-L-α-aminoglutarimide (III). In order to uncover the source of the amide formation the effects of various solvents, water and heat on (II) were studied. It was found that (II) is stable in purified dimethoxyethane, dioxane, and DMF; but the addition of water to the latter solvent caused a quantitative transformation of (II) to (III). Heat (70°) was also found to affect cyclization even in the absence of water. Contrary to earlier reports the formation of (III) was detected along with other products upon treatment of (II) with 2% triethylamine. The synthesis of (II) was finally accomplished in high yield by the DCCI method (0°, 3 hrs.) when the ester was isolated from isopropyl alcohol at 0°. In this context it is of interest that NPS-L-asparagine-OSu does not cyclize during isolation in the presence of water or during exposure to heat.

Neurohypophyseal hormones enhance the selective transfer of water and sodium ions across the wall of the toad urinary bladder. These processes are thought to depend upon the balance in activities of adenyl cyclase and cyclic nucleotide phosphodiesterase, the cellular enzymes which regulate the formation and degradation of 3', 5'AMP, respectively.

The problems of determining the lower and upper limits of that hormone-dependent adenyl cyclase activation which is translated into the permeability change was studied. The intrinsic hydroosmotic activities of oxytocin, 2-O-methyltyrosine-oxytocin (OMeOxy) and 2-O-ethyltyrosine-oxytocin (OEtOxy) were found to decline progressively (α=1.00, 0.88 and 0.00 respectively), corresponding to a change from an agonist to a partial agonist, to a competitive antagonist. While OEtOxy antagonized the action of oxytocin, it amplified the hydroosmotic action of theophylline, a phosphodiesterase inhibitor. The affinity constants for the synergistic and antagonistic receptors amounted to pS_2=7.00 and pA_2=6.85, respectively. The similarity in the binding constants for OEtOxy suggests to us that both processes, antagonism as well as synergism, originate from the same receptor system. This synergism is therefore a consequence of OEtOxy stimulation of the adenyl cyclase system, but the degree of this stimulation is insufficient for the triggering of a

(See Continuation Sheet)

16. Technical Progress in FY 1970: (Cont'd.)

hydroosmotic response in the absence of a phosphodiesterase inhibitor. The synergistic action of maximal concentrations of OEtOxy was mimicked by threshold concentrations of 3',5'-AMP. The inhibitory properties of PGE_1 were then studied. At maximal concentrations PGE_1 reduces the intrinsic activity of OMeOxy substantially from α=0.88 to 0.10; however, the intrinsic activity of oxytocin is only reduced from α=1.00 to 0.80. This non-competitive antagonism of PGE_1 reveals an excessive stimulation of the adenyl cyclase system by high concentrations of oxytocin. It may be concluded, therefore, that neurohypophyseal peptides are capable of producing graded effects on adenyl cyclase both below (subthreshold) and above (receptor reserve) the range of enzyme activity which evokes graded changes in membrane permeability.

The permeability to water of the urinary bladder of the toad Bufo marinus, can be increased with certain hypertonic serosal solutions. This response to hypertonicity was found to be similar in several respects to the hydroosmotic response of the bladdder following stimulation with vasopressin or cyclic 3' 5'-AMP. Relatively impermanent solutes, such as sodium and mannitol, are considerably more effective in promoting net mucosal to serosal water movement whereas highly permanent solutes, such as urea, are ineffective. While vasopressin inhibitors (prostaglandin E_1 and Mn^{++}) do not alter the response of the bladder to hypertonicity, cyclic 3',5'-AMP inhibitors (Ca^{++}, low K^+, pH 6.5) drastically reduce this response. It is concluded that there is an osmosensitive compartment, i.e. an osmoreceptor, in the toad bladder epithelium the activation of which triggers a hormone-like increase in the permeability to water of the tissue. It is suggested that this osmoreceptor exerts its effect after the formation of cyclic 3',5'-AMP in the chain of events which characterizes the action of vasopressin.

Progress toward the full understanding of the mechanism of sodium transport across living membranes was made by investigating the energetics of transport in the turtle bladder, a tissue with vigorous sodium transporting properties. The ratio of sodium ions moved across the epithelial layer to molecules of oxygen consumed was found to be 22, a value incompatible with the so-called "redox pump" mechanism. The value is of particular interest because it was derived from the epithelial layer only. The epithelium was separated from the underlying smooth muscle layer, the relative amounts and respiratory activities of the two types of tissue determined; the first time this has been done for a tissue used as a model for sodium transport. The two isolated layers of the turtle bladder are being investigated to determine the contribution of smooth muscle to phenomena previously attributed solely to transporting epithelial layers.

Studies were undertaken to determine the mode of action of the cardiac glycoside, ouabain, an inhibitor of sodium transport. To systematize metabolic effects that the picture of ouabain action must explain and to test the possibility that ouabain administration is synonymous with removal of external sodium, the response of oxygen consumption to ouabain

(See Continuation Sheet)

16. Technical Progress in FY 1970: (Cont'd.)

and to the removal of sodium was determined for four tissues whose ion-transporting activities have been well-characterized--cerebral cortex, urinary bladder, smooth muscle and gastric mucosa. Brain respiration was stimulated by ouabain, but inhibited by the substitution of choline for sodium, a finding incompatible with the frequently made assumption that ouabain administration and removal of sodium via choline substitution are basically identical procedures.

In the course of the work on oxygen consumption, information has been obtained on the Clark oxygen electrode. A new method of calibrating the electrode has been described, and problems in the measurement of respiration of isolated tissues brought to light and solved.

8-arginine-vasopressinoic acid, an analog of the mammalian antidiuretic hormone in which the carboxamide group of the glycine residue is replaced by a carboxyl group, was isolated after treatment of S-benzyl-N-tosyl-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-N^G-tosyl-L-arginyl-glycine-resin with anhydrous hydrogen fluoride, deprotection, oxidation and partition chromatography on Sephadex G-25 in the solvent system 1-butanol-ethanol-pyridine-0.1N acetic acid (4:1:1:7). This analog gave the expected values for elementary analysis and amino acid analysis. Upon testing for biological activity, 8-arginine-vasopressinoic acid was found to possess a high activity in the toad bladder hydroosmotic assay, but low activity in the rat uterotonic assay and no activity at a concentration as high as 3×10^{-5}M in the avian vasodepressor assay; most significantly, this hormone analog was found to inhibit AVP-induced antidiuresis in the rat.

Investigation of the substrate specificity of a partially purified enzyme from the toad bladder, which had been shown by Campbell et al, to cleave glycinamide from lysine-vasopressin, showed that this enzyme transgresses the boundaries of the substrate specificity for chymotrypsin as well as trypsin, because it catalyzes the cleavage of glycinamide from both oxytocin and lysine-vasopressin. Thus the amino acid residue in position 8 is not essential for the enzyme to recognize oxytocin and lysine-vasopressin as substrates, a contention which is further supported by findings here that the glycinamide moiety is readily cleaved from 3-phenylalanine-8-alanineoxytocin. However, the chemical functionality of the glycine residue in position 9 is of critical importance for the hormone to be recognized as a substrate. Only traces of glycine methylamide and glycine dimethylamide were detected upon incubation with enzyme of deamino-oxytocinoic acid methylamide and deamino-ocytocinoic acid dimethylamide, respectively. Oxytocinoic acid, an analog in which the carboxamide moiety of oxytocin is replaced by a carboxyl group was essentially unaffected. Attention was drawn to the possible general significance of enzymes--possessing a substrate specificity comparable or identical to that of 'carboxamidopeptidase' described in this study--in the overall regulation of the action of other peptide hormones in which the carboxyl-terminal amino acid residue bears a carboxamide group.

(See Continuation Sheet)

16. Technical Progress in FY 1970: (Cont'd.)

220 MHz proton nuclear magnetic resonance (NMR) was used in continued investigation of the conformation of neurohypophyseal hormones. This technique offers great promise in revealing information about helix → coil transitions and mobility of sidechains as well as intra- and intramolecular interactions in peptides and proteins. One prerequisite for a successful conformational investigation of a peptide or protein by NMR is the identification of the resonance pattern of individual protons of the constituent amino acids. Resonance peaks were assigned to specific residues in the neurohypophyseal peptides using: (a) examination of NMR spectra at different temperature levels; (b) comparison of spectra of intermediates of oxytocin and of analogs with selected structural modifications; and (c) homonuclear proton spin-decoupling. In the course of the assignment the variable temperature studies were particularly helpful in resolving overlapping resonance signals, while the spectra of intermediates and analogs give an indication of the chemical shifts and the resonance pattern to be expected for the individual amino acid residues in the hormone molecule. The decoupling experiments were vital for deciphering which NH, C^{α}, and C^{β} protons belong to a particular amino acid residue. With this approach, magnetic resonance peaks were assigned to individual protons of the constituent amino acids in the neurohypophyseal hormone, oxytocin. Some of the observed chemical shifts, and $NH\text{-}CH^{\alpha}$ coupling constants were studied in relation to the conformation of the hormone. (Schwartz, Walter)

17. Expected Results in FY 1971:

Some further improvements in the data-reduction capabilities of the on-line Sigma-2 programs are planned, but these are not major. A significant revision of the system to improve input-output facilities for processed data will be undertaken, because this is a present bottleneck. Access to Brooknet is anticipated this year, leading to an uncertain requirement for system modifications.

If it appears desirable in light of Brooknet operation, the CDC-6600 programs CONVOL and EPRSYN may be revised to take advantage of the Extended Core Storage facility. The postponed initiation of CDC-6600 program development for automatic analysis of EPR spectra may be undertaken depending on availability of collaborators.

The major progress anticipated with regard to experimental apparatus and instrumentation are: 1) the installation of the external magnetic field sweep control and its coordination with computerized data-taking, and 2) the setting up of ELDOR. As a new and complicated form of spectroscopy, ELDOR may require a major installation effort. A two-stage mixer may also need to be designed and fabricated for the Q-band EPR flow system.

The free radical studies on porphyrins will be active. The identification of the chlorophyll radical should be completed. Unusual oxidation states of some manganese and cobalt porphyrins will be pursued. A start

(See Continuation Sheet)

17. Expected Results in FY 1971: (Cont'd.)

will be made on experimental work with iron porphyrins, although many paramagnetic iron compounds do not exhibit EPR. ELDOR is strongly indicated for the porphyrin work and will be used, if available in time.

Computer-based EPR studies of the improved rhodopsin and rod-cell preparations should be completed. For lack of manpower committed to the project, it is not now clear whether the low-temperature liquid work or the charge-transfer studies will be resumed.

In order to improve old experiments on transient free radicals of catecholamine hormones, some of the critical flow experiments will be rerun to take advantage of the improved resolution and analytical capabilities of the computerized EPR system. Atom bombardment and irradiation of powders and crystals of purines, pyrimidines, and other nucleic acid components will continue. Structural analysis of the 9-methyl adenine radical should be complete. Correlation with radiomimetic reactions run in the EPR flow apparatuses should be actively underway.

Resynthesis of new materials should allow digitization of spectra and computer analysis of at least some pyrimidines, nucleotides and nicotinamides. Further extension of the work from substituted nicotinamides to nucleotides themselves and eventually to enzyme reactions will depend on opportunities to collaborate with Rogers (Medical College of Virginia). Resumption of active work on tissue EPR signals is planned. Resolution of large-sample cavity problems should be completed. Preliminary evaluation of the Q-band EPR will be carried out, and ELDOR will be tried, if it is available. Depending on the evaluation of these methods, the previously proposed systematic analysis of tissues may be started. (Borg)

It is hoped to accomplish the elucidation of the three-dimensional crystal structure of deamino-6-seleno-oxytocin. Conformational studies in solution will be continued with the purpose of arriving at the solution secondary structure.

Cyclase preparations of various hormone-responsive tissues will be developed in order to study the mechanism of action of the neurohypophyseal hormones at multiple levels of organizational complexity. In this context it is planned to develop a method for measuring total cyclic nucleotide levels in the presence and absence of agents which are known to alter membrane properties.

The mechanism by which hypertonicity alters the water permeability of the toad bladder will be continued and a study concerning the mechanism by which the steroid aldosterone potentiates the ADH-induced hydroosmotic response will be initiated. (Schwartz, Walter)

(See Continuation Sheet)

18. Expected Results in FY 1972:

Logical continuation of the projects proposed for FY 1971 is to be expected. Those projects presently receiving reduced attention because of insufficient personnel should be supported. ENDOR spectroscopy will be instrumented, if there is sufficient support.

EPR, ENDOR and--probably--ELDOR can be of great importance in structural analysis of paramagnetic molecular centers. Hence, investigation of a productive collaboration with the Center for Structural Biochemistry should be an objective for FY 1972, at the latest.

Paramagnetic shifts in NMR lines can provide useful information about free radicals that can be stabilized. Collaboration with an NMR group--preferably at BNL--is apt to be necessary by this time.

Spin labels are being used with increasing frequency in biochemistry and biophysics. They may have special application to structural studies. Consideration should be given to setting up a support facility for EPR of spin-labeled samples.

A conceptual review should be undertaken of the relevance of free radical biochemistry and of EPR and related spectroscopies to environmental problems. At this time the role of photochemical degradations involving free radicals in the fate and behavior of atmospheric pollutants would seem worthy of investigation, but it is unclear that this is a good subject for an investigator with a medical background. (Borg)

As many of the hormonal studies will be continued as collaborative support permits. (Schwartz, Walter)

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

Capital equipment required for progress in these studies include: ENDOR Accessory, $32,700 in FY 1971 and replacement EPR equipment, $40,000 in FY 1972.

20. Proposed Obligations for Related Construction, if any:

Bio-Med conjunct facility

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory — Laboratory

06-Biology & Medicine — Program

1. Contractor: Associated Universities, Inc.
Contract No.: AT-30-2-GEN-16
Task No.:

2. Project Title:
Radiation Genetics--Medical Research
189 No.: 06-165

3. Budget Activity No.: 06-01-03

4. Date Prepared: May 1970

5. Method of Reporting: See sub-activities

6. Working Location: Brookhaven National Laboratory

PRIVACY ACT MATERIAL REMOVED

7. Person in Charge: See sub-activities

Principal Investigator: See sub-activities

8. Project Term: Continuing
From: To:

9. Man-Years:	FY 1970	FY 1971	FY 1972
Sci., Res. Assoc. (Ph.D. or Equiv.)	3.0	3.0	3.0
Visiting Sci.	-	-	-
Prof. (B.S. or Equiv.)	-	-	-
Sci. & Eng. - Total	3.0	3.0	3.0
Technical	7.0	4.5	7.0
Adm. & Service	2.0	1.5	2.0
Guests & Research Collaborators	0.5	0.5	1.0
Total	12.5	9.5	13.0

10. Costs (In Thousands of Dollars):	FY 1970	FY 1971	FY 1972
Labor (including benefits)	163	141	166
Mats., Trav., Dev. Subcont., Spec'l Proc.	71	43	41
Reactor, Accel., and/or Computer Usage	0	0	0
Allocated Technical Services	2	1	1
Gen. & Adm. Overhead			
Total Research Cost			

PRIVACY ACT MATERIAL REMOVED

11. Reactor Concept:

12. Materials:

SUMMARY

Sub-Activity	Title
	Medical Research
06-01-03-(a)	Storage and Transfer of the Genetic Message

(See Continuation Sheet)

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory
Laboratory

06-Biology & Medicine
Program

1. Contractor: Associated Universities, Inc.
Contract No.: AT-30-2-GEN-16
Task No.:

2. Project Title:
Radiation Genetics - Medical Research
Storage and Transfer of the Genetic Message

189 No.: 06-167

3. Budget Activity No.: 06-01-03-(a)

4. Date Prepared: May 1970

5. Method of Reporting:
BNL Annual Report
BNL Monthly Letter to AEC
Bulletin of the Medical Department

6. Working Location:
Brookhaven National Laboratory

7. Person in Charge:
L. D. Hamilton

8. Project Term:
Continuing

Principal Investigator:
L. D. Hamilton
S. L. Commerford
N. Delihas

From:

To:

M.H.F. Wilkins & Associates (King's College, London)

9. Man-Years:

Direct Man-Years	FY 1970	FY 1971	FY 1972
Scientific & Professional	3.0	3.0	3.0
Other	9.0	6.0	9.0
Guests & Res. Collaborators	0.5	0.5	1.0
Total	12.5	9.5	13.0

10. Costs (In Thousands of Dollars):

	FY 1970	FY 1971	FY 1972
Research Division	275	237	247
Hospital Division	35	13	38
Research Costs	310	250	285

11. Reactor Concept:

12. Materials:

13. Publications:

Richmond, J. Y. and Hamilton, L. D. Foot and mouth disease virus inhibition induced in mice by synthetic double-stranded RNA (polyriboinosinic and polyribocytidylic acids). Proc. Nat. Acad. Sci. U.S. 64, No. 1, 81-6 (1969).

Hamilton, L. D., Babcock, V. I., Southam, C. M. Inhibition of herpes simplex virus by synthetic double-stranded RNA (polyriboadenylic and polyribouridylic acids and polyriboinosinic and polyribocytidylic acids). Proc. Nat. Acad. Sci. U. S. 64, No. 3, 878-83 (1969).

Wilkins, M. H. F., Wilson, H. R. and Hamilton, L. D. Secondary structure of DNA. Proc. Nat. Acad. Sci. U. S. (in press).

Wilkins, M. H. F., Arnott, S., Marvin, D. A., Hamilton, L. D. Fourier analysis and the structure of DNA: some misconceptions on Fourier analysis and Watson-Crick base-pairing. Science (in press).

14. Scope:

Short- and long-range objectives in this activity include research on the physical structure of storage and transfer of genetic information, and how a wide variety of chemicals damage informational macromolecules. Protein synthesis continues to be the central problem. Despite advances, e.g. coding and cell-free systems, it is likely to remain central for another decade--or at least until the structures of ribosomes and transfer RNA molecules, etc., are elucidated and protein synthesis is described accurately in 3-dimensions. Despite worldwide expansion in research on this subject, further nucleic acid-protein studies will be needed for several decades before intracellular processes in higher organisms are understood. Now that the chemical basis of coding is clear, interest is shifting to structural aspects of protein synthesis, e.g., as studied here in collaboration with the Medical Research Council's Biophysics Research Unit, King's College, London. The methods originally developed for DNA are now being deployed for RNA's and ribosomal structure.

In addition, biochemical studies of ribosomal structure, and how RNA segments interact with ribosomal proteins, are carried out on Escherichia coli ribosomes by (a) treating ribosomes with ribonuclease (RNase) to determine which RNA portions are vulnerable to cleavage and thus define their role in particle stability; and (b) labeling ribosomes in vitro with I-125 to determine which ribosomal components are accessible for labeling. The transcription from DNA to messenger RNA is studied by (a) detailing the 3-dimensional conformation of RNA and the interactions of synthetic polyribo- and polydeoxynucleotides; and (b) biophysical and biochemical characterization of the messenger RNA-DNA-RNA polymerase complex. Short-term objectives of this research are to determine (a) conditions for the reaction of iodine with nucleic acids so that iodine is bound at known positions, in the amount desired, with minimal side-reactions; and (b)

(See Continuation Sheet)

14. Scope: (Cont'd.)

the effect of this addition of iodine and its radioactive isotopes on the physical and biological properties of DNA and RNA. The availability of such a labeling procedure would assist in analysis of the transfer of information from DNA to various RNA's.

Study of the interaction of chemicals with the informational macromolecules needs extension and entails expanded research on the interactions of the macromolecules themselves and of the enzymes involved in their own synthesis and protein synthesis generally. These interactions are central to understanding how factors in the environment influence heredity and distort differentiation. They also relate to how viruses, chemicals and radiation induce somatic mutations, and how they and other factors accelerate aging.

A spin-off of the ability to make purified, synthetic, double-stranded RNA's and to monitor the extent of their double-helicity by x-ray diffraction, has been the practical tests of these materials as inducers of interferon in the treatment of several virus diseases and tumors in animals. These two-stranded complexes are being tested here and in collaboration with several investigators at Sloan-Kettering Institute for Cancer Research, New York: effects of poly rA:rU on herpes simplex virus (HSV) (with Dr. Chester Southam); the effects mainly of poly rI:rC on tumor inhibition (with Dr. G. Tarnowski and Dr. C. C. Stock); the effects on tissue culture and reticuloendothelial system activity and tumor inhibition (with Dr. Lloyd Old); and pharmacology of the complexes and the polynucleotides (with Drs. F. S. Philips, S. Sternberg, M. Fleisher, H. Marquart, and M. Zedeck). Collaboration with Plum Island Animal Disease Laboratory, U. S. Department of Agriculture, Greenport, New York, includes study of the effects mainly of poly rI:rC on foot-and-mouth disease virus (FMD) (with Drs. J. Richmond and J. W. McVicar). These studies on the biological effects of synthetic polynucleotides occasion some postponements of work on the study of the structure of informational macromolecules and of their interaction with small molecules and collaborative studies with the Biophysics Research Unit, King's College.

15. Relationship to Other Projects:

Storage and transfer of information is the central problem of contemporary biology. Related studies are being carried on in laboratories throughout the world.

Related studies at BNL include Burtin on ϕX DNA, Studier on bacteriophage T_7 DNA, and Lacks on the mechanism of bacterial transformation by DNA, all in the Biology Department.

Others working on structure of polynucleotides with x-ray diffraction include Alexander Rich at MIT, Cambridge, and David Davies at NIH, Bethesda,

(See Continuation Sheet)

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

(structure of synthetic polynucleotides); Robert Langridge, Biophysics Department, Princeton, (structure of ribosomes and viral RNA); and V. Luzatti at Strasburg, France, (structure of nucleoproteins). There is no identical work being carried on in any AEC laboratory

Kornberg at Stanford, Bollum at Kansas, and Khorana at MIT are studying the enzymic synthesis of DNA and synthetic deoxypolynucleotides. Dudock at State University at Stony Brook is determining the primary sequences of transfer RNA's. Sidney Brenner and Francis Crick at Cambridge, England are investigating coding by genetic experiments and the structure and detailed function of transfer RNA. Studies on the amino-acid code are being made with synthetic polyribonucleotides by Nirenberg, NIH, Bethesda, and Ochoa and co-workers at NYU, New York

Watson at Harvard and Cold Spring Harbor, Jacob and Monod at Pasteur Institute, Spiegelman at the Delafield, New York, are studying the transfer of information by messenger RNA, its relation to DNA and ribosomes and the interaction of various polynucleotides. Research on DNA-RNA hybrids is being done by Hayashi at La Jolla, Konrad at Berkeley and Spiegelman; they have examined virus infected cells for such hybrids with conflicting results.

Brammer at Berkeley and Zamecnik at MGH have investigated the effect of bromination on the infectivity of TMV-RNA and the amino acid accepting activities of yeast transfer RNA respectively. Brammer has also investigated to a minor extent the effect of iodination on the infectivity of TMV-RNA. However, the reaction conditions and the sites and extent of this iodination differed from those done here.

Nomura and Traub at University of Wisconsin are studying bacterial ribosome structure and function. Ribosomal RNA is being studied by Hadjiolov and co-workers in Sofia, Bulgaria, working on the overall primary structure of liver ribosomal RNA; Spencer, M.R.C. Biophysics Research Unit, London, is studying a crystallizable RNA fragment structure from bacterial and yeast ribosomes; Cox, Mill Hill, the secondary structure of reticulocyte ribosomal RNA; Midgley, England, E. coli ribosomal RNA structure; and Gould, Imperial College, polyacrylamide gel electrophoresis separation of ribosomal RNA fragments.

Other physico-chemical studies, e.g. melting curves to determine sequence complementary on nucleic acids are being made by Doty at Harvard and on transfer RNA's by Berg at Stanford. The Setlows at Oak Ridge National Laboratory (AEC) are studying enzymes that repair DNA after damage, and Freifelder at Brandeis, the nature of the lethal lesion in DNA after radiation injury. Increasing evidence from many laboratories is pinpointing the nucleic acids as key targets of radiation damage.

(See Continuation Sheet)

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

The effects of polynucleotides and of other interferon-inducers, especially the anti-viral and anti-tumor effects, are being studied in many laboratories. The main investigators include Field, Tytell, Lampson, and Hilleman at the Merck Institute, Baron and Levy at NIH, Merigan at Stanford, Colby and Chamberlain at Berkeley. Although some of these investigators work on closely related biological effects, the studies done here and at collaborating laboratories differ importantly in: (1) physical characterization of the polynucleotide complexes; (2) uniqueness of viruses studied, e.g. FMDV only at Plum Island; (3) uniqueness of experimental tumors tested at Sloan-Kettering; (4) comprehensiveness of pharmacology and toxicological studies at Sloan-Kettering.

16. Technical Progress in FY 1970:

Direct interest in DNA conformation has been virtually exhausted except for DNA's considered unusual by virtue of source, size, or base composition, and DNA's in which thymine is replaced by a halogenated uracil. X-ray diffraction studies to date do not support the concept that DNA consists of subunits linked by phosphopeptides. Were DNA composed of regular subunits, one should be able to get much more highly crystalline x-ray diffraction patterns from such subunits than earlier obtained from intact DNA. This expectation is based on the fact that ridding DNA of the phosphopeptide linkages and ending up with a family of identical subunits, these should pack much more regularly than the more heterogeneous DNA's. In studying fractions, the original 10S fraction gave a poor B pattern; the 6S fraction was no worse--if anything the pattern indicated slightly more crystallinity--but still poor. One concludes that dissociation does not produce good quality DNA since these smaller units only have the ability to give B patterns and not the more crystalline A patterns. The repolymerized 10S fraction also gave a very poor B pattern; again, the material was not able to pass into the A conformation, indicating that although the material consisted of double-stranded DNA, it was altered in some way so that it could not undergo the normal B to A transition. The poor quality of the pattern again indicates that the product was poor. In view of the fact that highly crystalline A patterns of DNA have been obtained with many DNA's of much higher molecular weight than either the original 10S fraction or the repolymerized 10S fraction, it would appear unlikely that the repolymerized material represents DNA in its natural state. Such poor patterns might be given by DNA in which there was some side-to-side aggregation rather than one continuous double-helix embodying the postulated links. In summary, the x-ray data to date speak against the concept of subunits in DNA.

Further studies have been completed on synthetic, double-stranded copolymers formed from a polyribonucleotide and a polydeoxyribonucleotide, i.e. polydeoxyriboadenylic and polyribouridylic acids, and polydeoxythymidylic and polyriboadenylic acids. Interesting patterns have been

(See Continuation Sheet)

16. Technical Progress in FY 1970: (Cont'd.)

obtained with the poly dAU complex. There has been difficulty in securing adequate polydeoxythymidylic acid; this has made diffraction patterns of poly dTA complexes unsatisfactory. Complexes of poly dAU have been observed in which there are two strands of polyribouridylic acid to one of polydeoxyriboadenylic acid; there were also duplexes in which there are single strands of poly dA and poly U. This is the first time that double-stranded copolymers have been observed in the complex formed from a polyribonucleotide and the polydeoxyribonucleotide. Moreover, in this conformation the duplexes are in the A conformation and there are shifts in conformation with addition of salt.

Crude RNA has been prepared from wound tumors as the first stage in the isolation of the double-stranded RNA from the wound-tumor virus. Improvement in the material isolated from the wound tumors has not been possible because of the diversion of time to the synthetic polynucleotide program; material has therefore been stockpiled for further purification. For similar reasons, little has been done on interaction of drugs with polynucleotides other than continuing work on papers on interactions of small molecules with DNA, RNA, and especially on the complex of daunomycin and DNA, on intercalation versus external binding by drugs in general to DNA, and on actinomycin and DNA.

In studies on structure and function of ribosomes it was found that digestion of 70S ribosomes with pancreatic RNase at 0^{o} in 10^{-2} M-Tris-HCL (pH 7.8), 10^{-2} M-$MgCl_2$, 3×10^{-2} M-NH_4Cl releases about 3% of the A_{260} units in low molecular weight form. RNA isolated from the intact ribosomes has no 23S component but predominantly 16-18S and 10-12S components. 70S particles do not dissociate after digestion under these conditions to 50S and 30S subunits, but do so if they are pretreated mildly with trypsin. RNase-treated particles are as active as undigested ribosomes in *in vitro* poly U-directed polyphenylanine synthesis; thus the activity of ribosomes was not affected by the presence of RNase-induced breaks in the RNA. 70S ribosomes can be readily iodinated *in vitro* with I-125 at 40^{o}C, pH 5.0, 10^{-2} M-$MgCl_2$. Over 95% of label bound to the ribosome is on the protein with a minor fraction on the RNA. All bands of 70S ribosomal proteins separated in polyacrylamide gel electrophoresis contain I-125 label. Ribosomal RNA in the free state labels readily under the same conditions of reaction. The RNA *in situ* appears largely unaccessible for labeling whereas many of the proteins are readily iodinated.

Conditions have been found which permit iodine to form a stable bond with the cytosine of RNA or DNA by substitution of the proton at C5. This reaction is specific for cytosine and no irreversible side reactions appear to occur since iodination, at least at low levels, does not affect the molecular weight, the ultra violet absorption, the melting temperature, the speed or extent of renaturation or the transforming properties of DNA. The reaction is very sensitive to the secondary structure of the nucleic

(See Continuation Sheet)

16. Technical Progress in FY 1970: (Cont'd.)

acid. Up to 30% of the cytosine of DNA have been iodinated and RNA has been labeled with the radioactive isotope of iodine I-125, at levels up to 1 μc/mg RNA.

In antiviral studies on biological effects of polynucleotides, synthetic 2-stranded poly rA:rU as well as poly rI:rC protected against systemic herpes simplex infection in mice, herpes simplex-induced cytopathogenicity in human cell line HEp2 tissue cultures, and herpes simplex-induced keratoconjunctivitis in rabbits. Both poly rA:rU and poly rI:rC were effective in vivo and in vitro and when given systemically or topically. Topically, the effect was localized in that if only one eye was treated with polynucleotide complex, the contralateral infected eye was unprotected. Furthermore, some rabbits whose eyes had responded to local treatment with the polynucleotide died of cerebral involvement with the virus--again indicating localization of the therapeutic response.

Herpes Simplex virus is a DNA virus. Protection by 2-stranded polynucleotide complexes extends to other kinds of viruses. Thus poly rI:rC induced host resistance to foot-and-mouth disease virus (FMDV--an RNA virus) when μg quantities were injected into mice. There was a graded response as shown by titrations of polynucleotide complex or virus. Protection was effective for > 48 hours after single injection of polynucleotide complex. Survival and serum interferon titers were directly related. The fact that poly rI:rC induced resistance to FMDV, while single-stranded unaggregated homopolymers of poly rI and poly rC did not, imply that a prerequisite for in vivo activity against virus is 2-strandedness. Preliminary experiments in guinea pigs and cattle have shown similar responses to the polynucleotide complex.

Effectiveness of the 2-stranded polynucleotide complexes in protecting against certain virus diseases led to their trial against experimental animal tumors. With some tumors the effects of the polynucleotide complexes have been inconstant and minimal, e.g. RADA1 leukemia in A mice. With some others and especially solid tumors, e.g. Ridgeway osteogenic sarcoma, the complexes have been consistently active. The mode of action of the antitumor effect of the polynucleotides remains obscure, but from present results it does not seem likely that the polynucleotides are acting against tumors by induction of interferon, via the reticuloendothelial system (RES), or by acting as an endotoxin-like substance.

RES activity, measured by the carbon clearance slope of mice given 20, 100, and 500μg per day i.p. of poly rI:rC for 7 consecutive days, did not increase; indeed there was a hint of depression of activity, perhaps because of a charge change on the surface of the macrophages. This absence of significant modification of the functional activity of the RES by poly rI:rC contrasts with other agents known to modify function of phagocytic cells and to heighten resistance to virus challenge. Not all

(See Continuation Sheet)

16. Technical Progress in FY 1970: (Cont'd.)

agents of course that increase phagocytic activity of the RES alter resistance to viruses.

In experiments on RADA1 tissue culture cells on the uptake of valine-H-3, poly rI:rC did not affect uptake at concentrations as high as 100 µg/ml over periods as long as 24 hr. At 20, 50, and 100 µg/ml there was a tendency for the cells to clump, but no effect on protein synthesis at any concentration ranging from 0.2-100 µg/ml in 3, 5, 6, and 25 hr. This remarkable result contrasts with the activity of various metabolic inhibitors--actinomycin, iodacetamide, puromycin, chloramphenicol, p-fluorophenylalanine, cyclohexamide, deoxyadenosine, 5-fluorodeoxyuridine, 1-β-D-arabinofuranosylcytosine, hydrocortisone 21-phosphate--in this system.

Toxicity studies in mice, rats, dogs, and monkeys are being carried out with Dr. F. Philips and his associates at the Sloan-Kettering Institute as a prerequisite for trying these specific polynucleotide complexes against cancers and serious virus diseases in man. Because of the variability in the biological potency of the polynucleotide complexes from batch to batch depending, it now appears, on variability in the individual homopolymers used for the complexes, a decision was made to prepare a single batch of ~ 40 gms poly rI:rC. Toxicity studies would then be completed on this material and the characterized complex used for clinical trials. This material has been prepared and studies for its characterization are beginning.

17. Expected Results in FY 1971:

It is hoped to complete papers on the high-resolution data and refinement of B DNA, on the complex of daunomycin and DNA, on intercalation versus external binding by drugs in general to DNA, and on actinomycin and DNA.

If diversion of time from the synthetic polynucleotide program permits, double-stranded RNA will be prepared from wound tumor virus if possible in milligram amounts. This double-stranded RNA is important for elucidating in detail the molecular properties of the double-stranded helical regions in RNA structure and defining how RNA itself can function as the primary genetic material like DNA. A point of especial interest is whether a native double helical RNA such as wound tumor virus RNA will undergo the transition from a 11-fold to a 12-fold double helix under suitable ionic conditions. This information is needed to understand how helical regions of RNA probably interact with each other at various stages in information transfer leading to protein synthesis.

Further characterization studies will be made on synthetic polyribonucleic acids poly rA:rU, poly rI:rC, and poly rC:rG, and the co-polymers of polyribonucleotides and polydeoxynucleotides as the latter become available. The studies on the polyribonucleotides will be directed

(See Continuation Sheet)

17. Expected Results in FY 1971: (Cont'd.)

primarily toward their biological activity in in vitro and in vivo systems as inducers of interferon and thereby as possible inhibitors of virus growth. Interest on the interaction of polydeoxy- and polyribonucleotides relates to their conformation as a possible way of understanding how information is transferred from DNA to RNA.

Present schematic, hypothetical concepts of ribosome structure show the "surface" largely composed of double-stranded RNA with single-stranded "hairpin turn" regions projecting outwards. (See Colter, McPhie and Gratzer, Nature 216, 884, 1967). Data here with pancreatic ribonuclease do not support this concept of outward projection of single-stranded looped regions. To ascertain whether the ribosome surface is largely double-stranded RNA, ribonuclease III enzyme (see Robertson, Webster and Zinder, J. Biol. Chem. 243, 82, 1968) which specifically degraded double-stranded RNA regions will be purified and used to determine the susceptibility of the RNA in situ. Labeling studies of the ribosome with I-125 will be continued. If only surface RNA (and only single-stranded RNA) is labeled by reaction with I-125 then the position of label in RNA molecules can be determined by using purified exonucleases. In this way the exposed portion or surface of single-stranded RNA can be located along RNA chains.

The position in the chromosome of genes responsible for the synthesis of transfer RNA and ribosomal RNA will be determined by labeling these RNA's with I-125 and incubating the labeled RNA with fixed cell smears under conditions where the RNA will hybridize with the complementary sequence of the nucleic acid of the gene which served as template for their synthesis. The location of these genes can then be visualized by autoradiography.

The effect of extensive iodination on the physical properties of DNA, for example the sedimentation coefficient, density, melting temperatures and x-ray diffraction patterns will be measured. The effect of iodination on two biological properties of DNA, its ability to transform cells and its ability to serve as a template for the synthesis of RNA will also be determined.

Further studies will be made on the effect of the polynucleotide complexes on FMDV in goats. These studies will include the effects of multiple rather than single doses of the complex, a comparison of routes of administration, e.g. intranasal, i.v., etc. The long-term immunological effects of protection against FMDV with polynucleotide complexes will be studied in mice and where appropriate also in goats. The effects of various molecules on the complexes will be tried in an effort to prolong the duration of their effectiveness in vivo in mice.

In collaboration with Dr. Jorge Chiraboga and Dr. Martinez Silva of the Puerto Rico Nuclear Center, San Juan, the effect of polynucleotide complexes will be studied on Trypanosoma cruzi, Schistosoma mansoni

(See Continuation Sheet)

17. Expected Results in FY 1971: (Cont'd.)

infections in mice, and on other protozoal infections as model systems become available in vivo and in vitro

Further studies will be carried out on herpes simplex virus in mice on the effects of graded doses of polynucleotides with especial interest in the effects of lower and less frequest doses. Similar studies will be carried out on Arboviruses in mice, e.g. West-Nile and on Marek's agent (a herpes-like tumor-causing virus in chickens) in chicks; efforts here to grow herpes virus in chickens have failed. The effects of polynucleotide complexes will also be studied on Rous and herpes simplex virus in baby rats.

The necessary toxicity studies in mice, rats, dogs and monkeys will be completed on the 40 gm batch of poly rI:rC along with characterization of the physical and biochemical criteria of the material. Clinical trials on these complexes will then be carried out at the Memorial Sloan-Kettering Center in New York and, if possible, at Brookhaven.

18. Expected Results in FY 1972:

The aim is to establish the various nucleic acid structures with the greatest possible accuracy. Since new information, e.g. models for the structure of transfer RNA, isolation of single segments of DNA (genes?), the production of synthetic polynucleotides, is continuously forthcoming, this continues as a rapidly developing and expanding area. Such structures and their interactions should not only give clues on protein synthesis but also on intracellular regulatory mechanisms in growth and differentiation and possibly even on memory storage in the brain. The interaction of new classes of chemicals with nucleic acids will be studied as they and personnel become available to see how they might act in biological systems.

If synthetic double-stranded RNA's continue to be effective inducers of interferon in such quantities as to have therapeutic benefit in vivo they will merit testing against a wide variety of viral diseases in animals and man. While it is clear that in the first instance such tests might be confined to established viral diseases, in the event that the synthetic polynucleotides prove to have no harmful side effects they would warrant trial in diseases in man where virus etiology is suspected though not proven. This might offer possibilities in the treatment of several malignant diseases. Additional effort is needed to develop nucleoside analogs for polynucleotide synthesis; such polynucleotides may be more potent.

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

Major operating expense for purchase of polynucleotides is estimated at $75,000 for FY 1971 and $100,000 for FY 1972. Capital expenditure in FY 1972 is planned for an optical rotary dispersion apparatus modified for circular dichroism ($50,000).

(See Continuation Sheet)

20. Proposed Obligations for Related Construction Projects, if any:

Bio-Med conjunct facility

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory — Laboratory

06-Biology & Medicine — Program

1. Contractor: Associated Universities, Inc.
Contract No.: AT-30-2-GEN-16
Task No.:

2. Project Title: Exposure to External & Internal Radiation--Medical Research
189 No.: 06-216

3. Budget Activity No.: 06-02-01

PRIVACY ACT MATERIAL REMOVED

4. Date Prepared: May 1970

5. Method of Reporting: See Sub-Activities

6. Working Location: Brookhaven National Laboratory

7. Person in Charge: See Sub-Activities

Principal Investigator: See Sub-Activities

8. Project Term: Continuing
From: To:

9. Man-Years:	FY 1970	FY 1971	FY 1972
Sci., Res. Assoc. (Ph.D. or Equiv)	6.5	6.5	6.5
Visiting Sci.	1.5	1.5	2.0
Prof. (B.S. or Equiv.)	1.5	1.0	2.0
Sci. & Eng. - Total	9.5	9.0	10.5
Technical	12.5	16.5	17.5
Adm. & Service	3.5	3.5	4.5
Guests & Research Collaborators	3.0	3.0	4.0
Total	28.5	32.0	36.5

10. Costs (In Thousands of Dollars):	FY 1970	FY 1971	FY 1972
Labor (including benefits)	383	406	456
Mats., Trav., Dev. Subcont., Spec'l Proc.	45	42	55
Reactor, Accel., and/or Computer Usage	16	16	17
Allocated Technical Services	22	22	24
Gen. & Adm. Overhead			
Total Research Cost			

PRIVACY ACT MATERIAL REMOVED

11. Reactor Concept:

12. Materials:

Project Title: Exposure to External and Internal Radiation--Medical Research 06-02-01

SUMMARY

Sub-Activity	Title
	Medical Research
06-02-01-(a)	Medical Studies of the People of the Marshall Islands Accidentally Exposed to Fallout. Antigenic Studies of Lymphocyte Culture
06-02-01-(b)	In-Vivo Measurement of Radionuclides in Man; Body Burden and Kinetic Factors. Computer Applications.

(See Continuation Sheet)

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory	06-Biology & Medicine
Laboratory	Program

1. Contractor: Associated Universities, Inc. | Contract No.: AT-30-2-GEN-16 | Task No.:

2. Project Title: Exposure to External and Internal Radiation-Medical Research Medical Studies of the People of the Marshall Islands Accidentally Exposed to Fallout. Antigenic Studies of Lymphocyte Culture | 189 No.: 06-218

3. Budget Activity No.: 06-02-01-(a)

4. Date Prepared: May 1970

5. Method of Reporting:
BNL Annual Report
BNL Monthly Letter to AEC
Bulletin of the Medical Department

6. Working Location: Brookhaven National Laboratory

7. Person in Charge: R. A. Conard

8. Project Term: Continuing

Principal Investigator: R. A. Conard; Y. H. Oh (AUI Fellow - NIH Grant)

From: To:

9. Man-Years:

Direct Man-Years	FY 1970	FY 1971	FY 1972
Scientific & Professional	2.5	2.5	3.0
Other	4.2	5.0	5.2
Guests & Res. Collaborators	2.0	2.0	2.0
Total	8.7	9.5	10.2

10. Costs (In Thousands of Dollars):

	FY 1970	FY 1971	FY 1972
Research Division	109	112	122
Hospital Division	31	33	38
Research Costs	140	145	160

11. Reactor Concept:

12. Materials:

Project Title: Exposure to External and Internal Radiation - Medical Research Medical Studies of the People of the Marshall Islands Accidentally Exposed to Fallout. Antigenic Studies of Lymphocyte Culture 06-02-01-(a)

13. Publications:

Conard, R. A. and Demoise, C. F. Preparation and purification of tritiated phytonemagglutinin and studies of cellular localization in human leukocyte cultures. Blood 35, 44-55 (1970). 1333

Conard, R. A. Quantitative study of radiation effects in phytohemagglutinin stimulated cultures. Intern. J. Radiation Biol. 16, No. 2, 157-65 (1969). 1361

Sutow, W. W. and Conard, R. A The effects of fallout radiation on Marshallese children. Radiation Biology of the Fetal and Juvenile Mammal, (Proc. of the 9th Annual Hanford Biology Symposium, Richland, Washington, April 1969), M. R. Sikov and Dennis Mahlem, Editors, pp. 661-73, U.S.A.E.C. Symposium Series, No. 17, Oak Ridge, Tennessee, 1969. 1355

Conard, R. A., Sutow, W. W., Colcock, B. P., Dobyns, B. and Paglia, D. E. Thyroid nodules as a late effect of exposure to fallout. Radiation-Induced Cancer, (Proc. of IAEA Symposium, Athens, Greece, April 1969), pp. 325-36, IAEA, Austria, 1969. 1356

14. Scope:

The primary objective in this activity is the determination of the lifetime effects of fallout radiation on the people of the Marshall Islands who were accidentally exposed to radioactive fallout in 1954. The 15th-year post-exposure medical survey, completed in March 1969, was carried out jointly by Brookhaven National Laboratory, under the auspices of the Atomic Energy Commission, and the Trust Territory of the Pacific Islands; collaborators from many institutions participate in the continuing studies. In addition to the exposed group, over 200 unexposed Marshallese are examined and serve as a "comparison population" in the assessment of possible late effects of acute fallout radiation. The observation of such late effects as lesions and growth impairment in exposed children fully indicate the need for examinations in the field to be as complete as possible along with additional laboratory studies in the U. S. Thyroid patients returned to the United States for surgical exploration receive complete hospital and laboratory studies. In addition to routine physical, hematological and other laboratory examinations, special ancillary studies related to aging, malignancy, reproduction, and body burdens of radionuclides are carried on. Reports of findings are published both in the open literature and as BNL reports.

A secondary objective in this activity is the elucidation of intracellular changes in cells responding to proliferative stimuli. Alterations are studied in two types of proliferating cell systems: a) human lymphocytes in culture stimulated with radioactively labeled phytohemagglutinin (PHA), a bean extract with potent mitogenic properties for lymphocytes; and b) in rat regenerating liver cells induced by partial hepatectomy.

(See Continuation Sheet)

14. Scope: (Cont'd.)

The intracellular changes (transformation) occurring in such cells is poorly understood. In lymphocytes, such changes are part of the immunological (antigen-antibody) response and of great importance in understanding the mechanisms involved in regenerating organs and tissues.

With achievement of the chemical purification, characterization and the determination of molecular structure of the mitogenic component of a radioactively tagged PHA with high specific activity, it is hoped that the antigenic nature of the mitogenic moiety may be explained on the basis of structural conformational characteristics and the precise intracellular localization determined. Localization and mechanism of action are studied by (a) autoradiography of transformed lymphocytes tagged with tritiated PHA; (b) differential centrifugation to obtain subcellular fractions from disrupted cells; (c) biochemical study of organelles and PHA protein-complexes; and (d) electron microscopy. In addition, PHA-stimulated lymphocytes are studied for in vitro radiation effect, response of leukemic cells and lymphocytes in other diseases and in senescence.

Parallel studies are conducted on the correlation of modification of nuclear histones with intracellular PHA action and cytoplasmic organelle functions and effect of aging on DNA template activity for RNA synthesis by structural modification of histones.

15. Relationship to Other Projects:

The studies of the exposed Marshallese are closely related to those of the Atomic Bomb Casualty Commission in Japan on the people of Hiroshima and Nagasaki and also on the studies of the 23 Japanese fishermen who were involved in the same accident as the Marshallese. Close liaison is maintained with Dr. George Darling and other collaborators at the ABCC and with Dr. T. Kumatori, who is in charge of the annual studies of the Japanese fishermen.

Related antigenic studies at BNL include those of Hamilton on delayed hypersensitivity and structural changes in lymphocytes in rats; Stoner on antigen-antibody complexes in stimulation of germinal centers of mice; and Cronkite on lymphopoiesis in human beings and animals treated with extracorporeal irradiation of the blood.

Elsewhere, related studies are carried on by Hirschhorn at NYU, Chessen at NIH, and Inman and Gowan at Oxford Studies here are unique since a radioactively labeled PHA is used as a tool to study the antigenic and mitogenic alterations of lymphocytes. Purification of phytohemagglutinin is studied by Takahashi at the University of Minnesota and Rigas at the University of Oregon Medical School. Although techniques developed by others are utilized, a rapid accurate method of assaying for mitogenic activity of various fractions by electronic sizing of cell nuclei is used here so that the purification procedures are greatly facilitated.

(See Continuation Sheet)

16. Technical Progress in FY 1970:

As a result of the 15th medical survey, five Marshallese were returned to the United States for study and surgery. Three of the five were found to have malignant thyroid lesions. Two of these, along with a malignant thyroid lesion previously reported, were in the more heavily exposed Rongelap group and strongly implicate a radiation etiology of thyroid malignancy. The third malignant thyroid lesion was in a Utirik woman. Because of the low dose of radiation received by this latter group, a radiation etiology seems unlikely for this individual. Twenty-one cases of thyroid nodularity have been detected to date in the Rongelap population; fifteen were found in children exposed at less than ten years of age. Two additional boys in this age group developed atrophy of the thyroid glands with hypothyroidism. Surgical exploration of eighteen cases of nodularity have been carried out in the United States and except for the three malignancies in the heavier exposed group the lesions proved to be benign adenomatous nodules. During the 1969 survey, slit lamp enumeration of lens defects was carried out on a number of the Marshallese examined. Analyses of these data for radiation effects are in progress.

As part of work under an NIH training grant in Gerontology, certain aging studies were completed. Transformation and division of lymphocytes in peripheral blood cultures resulting from phytohemagglutinin stimulation showed a significant reduction in response with increasing age. However, the irradiated Marshallese showed about the same response as that of the unirradiated group. Serum protein studies showed a significant increase in gamma globulin levels with increasing age. In this case the exposed population lagged behind the unexposed population suggesting a radiation effect. Immunodiffusion studies carried out by Dr. Fahey at NIH showed increased IgG levels with increasing age. Studies are still in progress on chromosome preparations from peripheral blood leukocytes of both exposed and unexposed Marshallese to see if age dependent chromosomal aberrations could be detected.

The impending return of the Bikini people to their home island which has a slight residual radioactivity necessitates instigation of a continuing program of monitoring of the internal body burdens of these people. BNL has been charged with this additional responsibility. During the past survey a visit was made to Kili island where the Bikini people now reside and a number of urine samples were taken for radiochemical analysis. These base line analyses showed very low levels of radionuclides.

The first phase of the study on purification and localization of tritiated phytohemagglutinin was completed. The results reported last year were extended with further purification of the mitogenic moiety from the radioactive bean extract. This labeled PHA was found to be largely localized in the cytoplasm. Subcellular fractionation studies showed that the mitochondrial fraction had the greatest activity. Specific radioactivity has been insufficient however to detect localization in

(See Continuation Sheet)

16. Technical Progress in FY 1970: (Cont'd.)

cell organelles by electron microscopic autoradiography. Thus, the main effort now is reexamination of techniques for tritiation of the purified plant mitogen in order to develop one that will produce higher specific radioactivity.

Studies of the radiosensitivity of PHA stimulated lymphocytes to graded doses of x radiation showed that radiation effects were much less pronounced on the ability of lymphocytes to transform than on the reproductive capacity of these cells.

The lymphocytes of patients with chronic lymphocytic leukemia were studied for response to PHA in regard to DNA and RNA synthesis using flash labeling with tritiated nucleotides before and after extracorporeal irradiation. The PHA stimulation of lymphocytes in these cases is also under study for the presence of virus-like bodies by electron microscopy. Preliminary results indicate what appear to be virus-like bodies in cytoplasmic vacuoles in the PHA treated leukemic cells and in normal cells which have been inoculated with subcellular fractions of the leukemic cells.

A few studies of subcellular transfer of a "mitogenic factor" from disrupted transformed lymphocytes which had been flash labeled with tritiated thymidine inoculated into allogenic non-PHA treated cultures of lymphocytes were done. Preliminary indications are that there may be some transfer of such material since the new blast forms that appeared in the recipient cultures were labeled.

17. Expected Results in FY 1971:

In view of the continued increase in incidence of thyroid lesions in the exposed Marshallese and the seriousness of the malignant thyroid lesions, thyroid examinations on a large number of unexposed Marshallese people are required in order to give a broader comparative base for spontaneous incidence of thyroid lesions in non-exposed Marshallese on islands not yet studied. This is required to evaluate the possible significance of appearance of thyroid lesions in the low exposure group such as on Utirik. Further base line radiochemical urine analyses for some of the Bikini people are planned.

With improved physical-chemical techniques it is hoped that a highly purified mitogenic factor can be isolated from the bean extract and conformational studies ascertained. New techniques also should increase the specific radioactivity of the mitogenic factor. Thus, it is hoped to achieve a more precise localization of the mitogen within the cells and successful electron microscopic visualization of labeled organelles. Nuclear histones in aging rats will be studied. Studies of the altered response of lymphocytes to PHA stimulation in cases of chronic lymphocytic leukemia will be continued in hopes that more refined electron microscopic identification of the virus-like bodies in PHA stimulated lymphocytes in

(See Continuation Sheet)

17. Expected Results in FY 1971: (Cont'd.)

this disease will be accomplished and subcellular transfer to normal lymphocyte cultures verified.

18. Expected Results in FY 1972:

Surveys of the Marshallese will be continued in accordance with findings during previous surveys.

In the lymphocyte culture studies, the interorganelle relationships of the cell in response to antigens and antibody production will be investigated. It is believed that the use of PHA will afford a valuable tool in the further study of various disease states and immunological capacity of individuals.

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

None

20. Proposed Obligations for Related Construction Projects, if any:

Bio-Med conjunct facility,

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory	06-Biology & Medicine
Laboratory	Program

1. Contractor: Associated Universities, Inc. | Contract No.: AT-30-2-GEN-16 | Task No.:

2. Project Title: Exposure to External and Internal Radiation - Medical Research In-Vivo Measurement of Radionuclides in Man; Body Burden and Kinetic Factors. Computer Applications | 189 No.: 06-224

3. Budget Activity No.: 06-02-01-(b)

4. Date Prepared: May 1970

5. Method of Reporting:
BNL Annual Report
BNL Monthly Letter to AEC
Bulletin of the Medical Department

6. Working Location:
Brookhaven National Laboratory

7. Person in Charge:
J. S. Robertson
S. H. Cohn
H. L. Atkins

Principal Investigator:
J. S. Robertson H. L. Atkins
S. H. Cohn E. P. Cronkite

8. Project Term:
Continuing

From: To:

9. Man-Years:

Direct Man-Years	FY 1970	FY 1971	FY 1972
Scientific & Professional	7.0	6.5	7.5
Other	11.8	15.0	16.8
Guests & Res. Collaborators	1.0	1.0	2.0
Total	19.8	22.5	26.3

10. Costs (In Thousands of Dollars):

	FY 1970	FY 1971	FY 1972
Research Division	409	401	457
Hospital Division	101	138	158
Research Costs	510	539	615

11. Reactor Concept:

12. Materials:

Project Title: Exposure to External and Internal Radiation - Medical Research In-Vivo Measurement of Radionuclides in Man; Body Burden and Kinetic Factors. Computer Applications 06-02-01-(b)

13. Publications:

Hauser, W., Atkins, H. L., Nelson, K. C. and Richards, P. Technetium-99m-DTPA: a new radiopharmaceutical for brain and kidney scanning. Radiology (in press).

Hauser, W., Atkins, H. L. and Richards, P. Lymph node scanning with ^{99m}-Tc-sulfur colloid. Radiology 92, No. 6, 1369-71 (1969).

Cohn, S. H., Dombrowski, C. S., Pate, H. R. and Robertson, J. S. A whole-body counter with an invariant response to radionuclide distribution and body size. Physics in Medicine and Biology 14, No. 4, 645-50 (1969).

Cohn, S. H., Dombrowski, C. S. and Fairchild, R. In-vivo neutron activation analysis of calcium in man Intern. J. Applied Radioisotopes (in press).

Cohn, S. H. and Dombrowski, C. S. Absolute measurement of whole-body potassium by gamma-ray spectrometry. J. Nucl. Med. (in press).

Atkins, H. L., Hauser, W. and Richards, P. Factors affecting distribution of technetium-sulfur colloid. J. Reticuloendothelial Soc. (in press).

Hauser, W., Atkins, H. L. and Richards, P. Five years of experience with ^{99m}-Tc-sulfur colloid. N. Y. State J. Med. (in press).

Scherrer, J. R. and Cohn, S. H. Formulation of a stochastic model of long-term strontium retention data using the likelihood function. Biometrics (in press).

Krohn, D. L., Brandt, R., Straub, R. F. and Robertson, J. S. Intraocular heavy particle radiation of experimental malignant melanoma in rabbits. Am. J. Opthalmol. (in press).

14. Scope:

The studies reported in this budget activity are aimed at several broad objectives: the determination of the physiology of biologically active compounds and metabolites in order to gain information on the metabolism and toxicity of radioactive elements; the development and application of tracer theories to physiological studies; the development of methods for determining location and kinetics of isotopes and for the utilization of isotopes in diagnosis and therapy; and the development of necessary data processing and computer programming techniques for these studies and the other activities within the Medical Research Program.

A long-range objective is the elucidation of the kinetics of skeletal metabolism, as related to various states of disease and health. Four aspects of this program are studies on kinetics of calcium metabolism, whole-body

(See Continuation Sheet)

14. Scope: (Cont'd.)

counting, in vivo neutron activation analysis, and mathematical modeling.

Calcium (Ca) and strontium (Sr) metabolism are described using mathematical models which provide a reference for objective, reproducible measurements of subtle changes in the metabolic parameters of these elements under various conditions. The data on which the models are based were obtained from kinetic studies employing the tracers Ca-47, Sr-85, and F-18; from whole-body counts; and from measurements of whole-body calcium by in vivo neutron activation. The parameters of metabolism measured are the rate of Ca resorption and accretion, and the size of the postulated exchangeable and nonexchangeable Ca "pools". Disorders of metabolism associated with immobility, weightlessness, hormonal imbalance and osteoporosis, conditions which are characterized by a loss of Ca from the skeleton, are studied. The study of osteoporosis provides data not only on the state of Ca metabolism associated with this condition, but also furnishes inferences on the homeostatic control of Ca deposition and resorption in bone. Therapeutic efforts to counteract the osteoporotic condition have consisted of Ca supplementation and administration of sodium fluoride.

The paramount need for the in vivo measurement of absolute levels of internally-deposited radionuclides occurs in the diagnosis of cases of accidental intake of radionuclides. It is also essential in numerous clinical studies (such as neutron activation analysis). Effort is directed toward minimizing the variations in counting efficiency introduced by changes in the spatial distribution of internally-deposited radionuclides, and by changes in the energy absorption as a function of body weight and habitus. The BNL whole-body counter is a multidetector system with two banks of counter-balancing detectors designed to minimize the above mentioned problems. In addition, the counter is linked to a computer programmed to introduce additional corrections for these factors. Hence, the BNL counter is the first and, to date, the only whole-body counting system which can provide directly an absolute, in vivo measurement of a radionuclide internally deposited in a human being.

Clinical studies in collaboration with BNL staff and others which previously were not amenable to investigation because of the necessity of absolute measurements or spatial localization of the radionuclide include the following: measurement of absolute levels of whole-body potassium and determination of its spatial distribution in the body; pharmacokinetics of halothane-Br-82 in man; measurement of lean-body mass in grossly obese children, based on the measurement of K-40; alterations in Mn-56 distribution resulting from the treatment with L-Dopa of patients with Parkinson's disease; short-term distribution and turnover of Tc-99m-DTPA chelate in man; measurement of levels of Cs-137, K-40 in 450 BNL staff members (variations with time and as a function of sex have been investigated); measurement of internal contamination in individuals involved in accidental exposures; calibration procedures for absolute measurements, and computer programs associated with such measurements; and the standardization of tests for the absorption of iron and vitamin B_{12}.

(See Continuation Sheet)

14. Scope: (Cont'd.)

In calcium kinetic studies it is extremely useful to determine the absolute value of the Ca concentration in the body (in contrast to determining the relative change). A technique of measuring Ca by in vivo neutron activation has now been sufficiently well developed so that it can be employed in study of osteoporosis.

Evaluation of the radiation hazard from Sr-90 requires information on the biological turnover rate of Sr in man. Since the biological behavior of Sr has been shown to be closely related to that of Ca, its role can readily be considered in terms of comparative kinetics. Improvement in the mathematical models to describe primarily the long-term retention of Sr in man is a main objective. (Cohn)

In the continuing work on the development of radiopharmaceuticals and their application in clinical studies, present effort centers on labeling of various compounds with Tc-99m with special emphasis on colloidal materials for marrow and lymph node examination Positron imaging with C-11, F-18, and Fe-52 compounds is of interest and collaborative work with the BNL Chemistry Department is carried on, particularly organic syntheses. Recoil labeling and rapid separation of organic compounds also is under investigation. Absorption edge spectroscopy utilizing monochromatic x-ray beams in a scanning apparatus is developed. With this method it is possible to delineate the distribution of an element within the body with rather high resolution. (Atkins)

To a large extent the mathematical and biophysical applications considered in this budget activity are ancillary to projects whose main features are described in other activities, or are supportive of general features of the Medical Research Program. These include equipping, maintaining and operating such service facilities as the whole-body counter and the central counting rooms, the instrumentation development laboratory, data processing assistance, and computer programming. Several independent projects are directed to the development of equipment for radiation detection and of computer programs for specific problems in radiation dosimetry and the analysis of tracer data. (Robertson)

Studies previously reported in budget activity 06-03-01-c (on vitamin B_{12}, iron absorption, and exocrine pancreatic insufficiency) will be reported in this activity as of FY 1971. (Cronkite)

15. Relationship to Other Projects:

Related kinetic studies of Ca metabolism have been performed by Bauer (Hospital for Special Surgery) and Bronner (University of Louisville); mathematical models similar to those developed here have been used by Pak (NIH). Investigators who have devoted considerable effort to minimizing the variations in counting efficiency in whole-body counting include:

(See Continuation Sheet)

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

Dudley (IAEA), Marinella (ANL), Naversten (Lund), and Genna (MIT). The BNL counter is the only system, however, which can correct simultaneously for both the spatial distribution and energy absorption of radionuclides by the body.

Representative of groups working on related clinical studies include those of: Pak (NIH), use of thyrocalcitonin in treatment of hypercalcemia of parathyroid carcinoma; Spencer (Hines Veterans Administration Hospital), effect of Na fluoride on Ca metabolism; Maletskos (Harvard), reliability and intercomparison of techniques in the measurement of Cs-137 in human beings; also, Anderson (LASL), Remenchik (ANL), Forbes (Rochester), and Burkinshaw (Leeds) have all measured whole-body K with high precision, but not accuracy.

Two other groups are presently involved in whole-body Ca measurement by neutron activation: Chamberlain (Birmingham), and Nelp-Palmer (University of Washington). Studies here have gone further by achieving not only a precision, but also a high accuracy in Ca measurement.

Mathematical models describing long-term turnover of Sr in man have been proposed by Marshall (ANL) and Rundo (Harwell). The present computer code employing a Causal Path Analysis and the Likelihood Function is a new approach; it is somewhat related to the "Random-Walk" hypothesis of Wise (King's College, London). (Cohn)

Investigations involving radiopharmaceuticals and cyclotron-produced nuclides are being carried out at many institutions. (Atkins)

Leaders in the field of development of instrumentation for locating brain tumors through the use of radioactively-labeled substances include: Anger (University of California, Berkeley); Bender (Vanderbilt University); Kuhl (University of Pennsylvania); and Brownell (MIT and Massachusetts General Hospital). Each has developed a different system which has established practical value. The multidetector positron system in development here differs from these in several basic ways, notably the provision of multiple pairing of small detectors which provides a higher quantum utilization than the other methods and offers a more efficient method for dynamic studies of cerebral blood flow as well as tumor localization.

Prince at BNL; Oliver at M. D. Anderson Hospital; and Stoddard at Savannah River are concerned with the dosimetry of californium-252 applicators. A computer program for computing the total neutron and gamma dose rates at selected lattice points in tissue was independently-developed here, tailored to the characteristics of the sources being used locally and in agreement both with theoretically-derived dose rates and with measured values.

Berman and Weiss at NIH originated the basic program in use for analysis of tracer data. The extent to which the applications here are

(See Continuation Sheet)

Cancer and Other Clinical Research - Medical Research
The Effects of Hormones, Drugs, and Nutritional Changes on the
Project Title: Intermediary Metabolism of Carbohydrates and Lipids 06-03-01-(f)

16. Technical Progress in FY 1970:

As previously described, in collaboration with Dr. Brodoff, L-alanine-U-C-14 was given intraperitoneally to fasting Egyptian "sand rats" with varying degrees of carbohydrate intolerance which can be ameliorated by electrolytic lesions of the arcuate nucleus of the hypothalamus and by the anti-adrenergic drug, tetrabenizine. Conversion of alanine-C-14 to blood glucose was measured as an index of gluconeogenesis. The range of conversion was from 2 to 10% among all rats and there were no significant differences between non-treated rats and those with lesions of the arcuate nucleus or treated with tetrabenizine, which suggests that the mechanism of improvement of glucose tolerance is not by decrease of gluconeogenesis. Further studies directed toward elucidation of effects on glycogenesis and glycogenolysis were done with glucose-6-C-14 and are currently being assayed.

For the study of effects of hydrocortisone on gluconeogenesis from malate-C-14, H-3 in perfused rat liver, some final samples of blood glucose were analyzed to test in vivo vs. in vitro effects of hydrocortisone. Hydrocortisone has caused increased gluconeogenesis from malate both in vivo and in vitro while longer action in vivo promotes glycogenesis. A manuscript was prepared on studies carried out in Sweden on conversion of pyruvate-2-C-14 to breath $^{14}CO_2$ and blood glucose in acromegalic patients. Patients with decreased glucose tolerance displayed also a reduction in rate of oxidation of pyruvate-2-C-14 without change in gluconeogenesis from pyruvate.

In the study of conversion of glucose-1-C-14 to $^{14}CO_2$ during glucose tolerance test, the oxidation of the labeled glucose during standard vs. cortisone glucose tolerance tests were compared in seven obese or obese, diabetic patients. While the plasma glucose concentration (1 to 2 hours after oral load) was increased about 10% on the average by cortisone, the conversion of glucose-1-C-14 to $^{14}CO_2$ was increased by 20% at 1 hour and 10% at 2 hours. This suggests that the short-term hyperglycemic effect of cortisone in vivo in man is predominantly due to an increase of gluconeogenesis rather than a decrease of utilization of blood glucose. Glucose-1-H-3 was also given to some of these patients and glucose-2-H-3 to others. Usually the rate of conversion of H-3 to body water was also increased by cortisone, but the results were more variable than with C-14. Glucose-2-H-3 was converted to ^{3}HOH distinctly faster than glucose-1-H-3 and possibly by a two-phase process during the first 3 hours.

Since increase of plasma fucose has been reported to be excessive in latent diabetes, some plasma samples taken from patients during glucose tolerance test with glucose-1-C-14 were analyzed by gas chromatography of volatile carbohydrate derivatives to measure fucose and isolate it for radioactivity analysis. No increase of fucose during the glucose tolerance test was found.

Cancer and Other Clinical Research - Medical Research
The Effects of Hormones, Drugs, and Nutritional Changes on the
Project Title: Intermediary Metabolism of Carbohydrates and Lipids 06-03-01-(f)

16. Technical Progress in FY 1970: (Cont'd.)

In the study of metabolism of glycerol-C-14, H-3 in obese, diabetic patients, comparison was made of the incorporation of tracer into plasma glycerides of these patients (6 females) vs. a group of 4 normal females. There are no significant differences between the groups for percent dose in total plasma triglycerides (TG) or di-+ mono-glycerides (DMG). For each group there is slightly more activity in the DMG than the TG. For the total glycerides the mean percent dose incorporation is less than 0.2%. Glycerol-C-14 and glycerol-H-3 show the same results, so there is no metabolic loss of H-3 before glyceride formation.

For the study of lipogenesis from different C-14-labeled saccharides (fructose, glucose, and sucrose) in normal rats, more accurate and detailed analyses have shown that the labeling of several types of lipids (glycerol and fatty acid moieties of triglycerides and di- + mono-glycerides, free fatty acids) of liver or plasma is 3 to 10 times higher from sucrose-C-14 than from glucose-C-14, with fructose-C-14 generally intermediate. This emphasizes a synergistic effect between glucose and fructose (the two moieties of sucrose) or else reflects more rapid absorption of the disaccharide (concomitant with hydrolysis by mucosal sucrase) than of either monosaccharide. Presently some lipid samples from rats given a mixture of labeled glucose and fructose (with an oral mixed load) are being analyzed to test the latter hypothesis.

Studies with C-14-labeled sucrose in patients have not yet been started, but non-isotopic sucrose tolerance tests in obese patients with analyses of the plasma curves of glucose and immunoreactive insulin (IRI) were done. Some patients seem to have higher insulin curves together with lower glucose curves than during glucose tolerance tests. This suggests special effects of sucrose and may help explain the high lipogenic potential of sucrose.

Studies initiated in patients using another labeled carbohydrate, galactose-1-C-14, which for theoretical reasons (particular inhibition of its oxidation by high reducing potential in the liver) could show differences in rate of formation of $^{14}CO_2$ between normal subjects vs. obese or diabetic patients greater than with labeled glucose. Study of one non-obese and three obese female patients so far are promising in this regard.

In the study of production and release of insulin by isolated rat pancreatic islets perfused by continuous flow of medium with serial analysis of IRI, the parameters of response to graded increase of glucose concentration and different initial and constant glucose concentrations were measured. Multiple major phases of release with fine peaks within the major cyclic release were revealed. Thresholds of response and saturating concentrations of glucose were defined and an initial paper has been submitted for publication.

(See Continuation Sheet)

Cancer and Other Clinical Research - Medical Research
The Effects of Hormones, Drugs, and Nutritional Changes on the
Project Title: Intermediary Metabolism of Carbohydrates and Lipids 06-03-01-(f)

17. Expected Results in FY 1971:

Analyses of C-14 in glycogen from liver of Egyptian "sand rats" given glucose-6-C-14 will be completed to test whether the anti-adrenergic drug, tetrabenizine, improves glucose tolerance in this species by an effect on glycogen metabolism. If adequate personnel is available, the question of gluconeogenesis from L-alanine-U-C-14 might be reexamined with administration of the labeled compound during an oral glucose load.

The studies of oxidation of glucose-1-C-14 and glucose-1-H-3 to $^{14}CO_2$ and ^{3}HOH in obese, diabetic patients will be continued without the prior administration of cortisone before the glucose tolerance test. To the same patient (and a matching set of normal subjects) will be given galactose-1-C-14 in an oral galactose load to compare with oxidation of labeled glucose.

Studies of formation of liver and plasma lipids of normal rats from sucrose-U-C-14 compared with glucose-U-C-14 + fructose-U-C-14 will be further pursued to delineate reasons for particular lipogenesis from sucrose. The plasma insulin response of these rats to the different carbohydrate loads may also be studied.

A beginning will be made on study of lipogenesis from sucrose in patients given sucrose-U-C-14 in an oral load. This may be compared with glucose-U-C-14 (or glycerol-2-C-14) in the same patient. Further, if additional personnel are available, there may be a study of the effect of different oral contraceptives on the metabolism of labeled carbohydrates to plasma lipids and rate of turnover of the latter. This should help explain mechanisms of recently observed effects of estrogens in contraceptives to cause glucose intolerance, hyperlipemia, and hyperinsulinemia and could indicate possible synergistic effects of high sucrose diet and mixed contraceptives. Using fractionation of insulin on sephadex gel and/or disc-gel electrophoresis, it is hoped to study the output of insulin and its variants (e.g., proinsulin) into plasma of these patients during carbohydrate load and into the medium of perfused rat pancreatic islets.

18. Expected Results in FY 1972:

The studies will be consolidated and reported with studies in 06-03-01-(b)

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

See 06-03-01-(b)

20. Proposed Obligations for Related Construction Projects:

See 06-03-01-(b)

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

unique depends on the current problems and the mathematical models used to analyze the data.

Nims at BNL Biology Department; Ussing at the University of Copenhagen; Tosteson at Duke University; Chinard at the Johns Hopkins University; Polson at the University of Capetown and many others have been concerned with theoretical and experimental aspects of membrane transport. The study here is directed to elucidating the relative roles of diffusion and hydrostatic pressure in osmotic flow across artificial membranes. (Robertson)

16. Technical Progress in FY 1970:

Osteoporosis is the manifestation of an altered dynamic equilibrium of bone mass. As a result of the Ca kinetic studies, a unified view of osteoporosis is developing, centered around the concept of Ca homeostasis. It appears that bone formation and resorption are homeostatically linked to maintain the Ca plasma level. The bone resorption rate appears to be highly sensitive to changing levels of plasma Ca through the effect of the Ca level on parathyroid hormone and thyrocalcitonin (TCT). Fluoride is used in the treatment of osteoporosis as its effect on bone is to render it more resistant to osteolytic forces. To date, studies on the effects of fluoride have been initiated in five patients with osteoporosis.

Study of the therapeutic effects of commercially available TCT on osteoporotic patients, initially delayed because medical personnel were not available for participation in the clinical aspect of the program, was further delayed by an FDA decision halting new field trials of TCT. A study on the effects of TCT on calcium kinetics in rats was completed, however, and results are being analyzed.

In collaboration with Bateman on the effects of chemotherapeutic agents on skeletal metabolism, comparative Ca-47, F-18 studies and neutron activation analysis were initiated on two patients with neoplastic disease.

A procedure for the measurement of the absolute level of body potassium by gamma ray spectrometry was developed with an accuracy of ± 3.65% in a population whose body weights ranged from 66 to 145 kg. Further, the spatial distribution of natural K-40 and injected K-42 was compared after correction for attenuation of the gamma photons. This measurement is clinically useful, since there is a progressive loss of K in severe disease. The levels of K-40 were measured in 450 BNL employees and plotted as a function of age and sex.

The pharmacokinetics of halothane-Br-82 and Br-82 gas were studied in five subjects. The novelty of the approach used resides in the preparation of Br-82-labeled halothane in the Medical Research Reactor (MRR) and its use in conjunction with the whole-body counter. The spatial distribution of the rapidly-translocating gas was measured in various segments

(See Continuation Sheet)

16. Technical Progress in FY 1970: (Cont'd.)

of the body following inhalation. A special respiratory system was used to administer and remove the expired gas with the subject in the counter. With a computer system it was possible to record 110 whole-body counts from one subject in the first 3 hours after inhalation. A mathematical model consisting of three compartments was assumed. The data obtained from various combinations of detectors were fitted to the model.

On the assumption that the K content of the body cell mass is constant, it is possible to estimate the body cell mass by measurement of K-40 with the whole-body counter. In collaboration with Knittle, Mt. Sinai School of Medicine, measurement was made of lean-body mass (K-40) in 35 grossly obese children and their siblings. There is evidence suggesting that Mn^{++} may be implicated in the mechanism of action of L-Dopa in the treatment of Parkinsonism. A study was initiated to measure the alteration in Mn^{++} distribution following Dopa therapy. Mn-56 was produced in the MRR and administered to two patients to ascertain if a changed pattern of Mn^{++} distribution and turnover after L-Dopa administration yielded clues on the mechanism of action of L-Dopa. In another intradepartmental collaboration (Atkins and Hauser), the distribution and turnover of Tc-99m-DTPA chelate administered i.v. was determined in nine patients. The average coefficient of variability of the 16 whole-body counts in each patient was ± 1.2% when correction for excretion was made.

The level of Cs-137 also was measured in the 450 BNL employees reported above as measured for K-40 as part of their routine physical examinations. In addition, a selected population of 10 medical staff members has been monitored for Cs-137 monthly over a period of nine years. These studies are valuable in that they reflect the Cs-137 in the environment as a result of fallout. In collaboration with Distenfeld of the BNL Health Physics Division, the whole-body counting and computer analysis techniques were used to identify and to estimate the absolute levels of a number of internally-deposited radionuclides in seven BNL staff members accidentally exposed to a uranium target in January 1969.

Considerable effort was put into the improvement of the absolute calibration of the whole-body counter, and into the development of new techniques for automatic data handling and preliminary analysis of the whole-body counting data. Since January 1969, a total of 5,062 whole-body counts has been collected and stored on magnetic tape.

With the present technique of in-vivo neutron activation of whole-body Ca, it is possible to measure the calcium in human beings with an estimated accuracy of ± 4%. The dose to the patient per analysis has been reduced to 0.63 rem (RBE = 10) from 14-Mev neutrons. To date 13 in-vivo measurements have been made on 7 patients (two with metastatic lesions and five with osteoporosis). Initial calibration studies have been carried out for the measurement of absolute levels of Na, Cl, and

(See Continuation Sheet)

16. Technical Progress in FY 1970: (Cont'd.)

N with the use of the same neutron generator and exposure technique.

Lack of funds and personnel has curtailed development of a facility for use of a 238 PuBe source of fast neutrons near the whole-body counter.

The stochastic model formulated and applied to the long-term Sr-85 data in rats was further developed and applied to the analysis of long-term (1 year) Sr-85 data previously obtained by the whole-body counting of 8 men. The retention data were analyzed first by means of a Causal Path Analysis, and then with the use of the Multiple Process Law a stochastic model was constructed. The parameters of the retention function and their statistical significance were estimated with a Maximum Likelihood Function by means of an iterative code (LIKELI). (Cohn)

Distribution studies in mice were used to further characterize the technetium-sulfur colloid. Several factors including dose, preloading and use of gelatin were noted to increase marrow deposition at the expense of liver localization. Attempts at affecting distribution in humans by pretreatment with gelatin were not successful. Lymph node scanning with Tc-sulfur colloid was carried out on a number of patients and proved quite feasible. Attempts are being made to increase distribution to higher echelon nodes by utilization of gelatin, hyaluronidase and "cold" colloid. Intraperitoneal administration has been utilized to demonstrate mediastinal nodes.

A series of colloidal materials labeled with Tc-99m was studied in mice and rabbits and some appear to be useful for lymph node and marrow studies.

C-11-labeled dopamine was synthesized but the specific activity was not high enough for practical use. F-18 labeling experiments to produce labeled amino acids and improved bone-seeking formulations were initiated.

Investigations of Tc-99m as TcO_4^- for thyroid studies continue with I-131 correlation studies and evaluation in thyroiditis patients.

Transmission scanning utilizing absorption edge spectroscopy has been demonstrated in a satisfactory manner with preliminary apparatus. A silicon diode detector was constructed and is being installed.

Low specific activity C-11-dopamine has been synthesized that has little practical application. Synthetic procedures are being revised with the aim of obtaining high specific activity C-11-dopamine. So far attempts to synthesize C-11-L-Dopa have failed. F-18 phenylalanine has been successfully prepared. Initial animal studies indicate it will be useful in clinical diagnosis because of its concentration in organs difficult to detect. (Atkins)

(See Continuation Sheet)

16. Technical Progress in FY 1970: (Cont'd.)

The requirements for californium-252 dose rate calculations developed more than had been fully anticipated, and the programming effort for this project has been correspondingly greater. The result is a program which takes into account the effects of the source tube wall thickness and end plug thickness as well as the geometry and attenuation by absorption in intervening tissue. To a large degree these problems are similar to those which have been worked out in some detail for radium needles (Quimby, Johns, Greenfield, etc.), but the presence of neutrons in the californium-252 emission requires somewhat different considerations than are applicable to gamma dosimetry. In particular, the attenuation by the source holder, while very important for gammas, is negligible for neutrons and the reactions with tissue involve different mechanisms. Instead of the customary method of using Sievert integrals, it has been found more satisfactory to achieve the required integrations by direct application of Gauss' numerical integration method to the expressions describing the gamma and neutron fluences as functions of angle relative to the source axis and distance from the source. The output includes a tabulation of the dose rates at up to 21 x 21 lattice points in a given plane and for up to 21 planes and an analogous type of representation of the same information but using a series of 32 graded intensities of printed characters (adopted from the method of Mendelsohn and Prewitt) instead of numerical values to represent the dose rates. Over 30 proposed arrays involving up to 32 radiation sources each have been computed by this method for use in analyzing experimental results obtained in irradiating pig's skin and tissue cultures, and in the development of arrays for projected use in patients. (Atkins)

The Sigma 2 computer system for time-shared operation of the whole-body counter and the Electron Paramagentic Resonance Spectrometer system was developed to a fully operational state. Modifications were made to both experimental equipment interfaces to facilitate more efficient and sophisticated operations. Programming for Sigma 2 applications continued to be principally concerned with these two problem areas. A second magnetic tape transport, more core memory, an external interrupt feature and the Brocknet interface were delivered. Work was begun to prepare an improved system so that additional users can be accommodated.

The program ANY, developed for computing kerma rates for neutrons produced by bombarding various targets with proton or deuteron beams at the Van de Graaff accelerator, was improved and expanded somewhat, but there were no major changes from the program described last year. A BNL report describing the program was published.

The EPRSYN program for simulating EPR curves has continued to be refined. However, the anticipated development of this as a basis for a data-fitting procedure has not been undertaken, partly because the success of the on-line procedures for data averaging and curve resolution enhancement have made the curve-fitting requirement less urgent.

(See Continuation Sheet)

16. Technical Progress in FY 1970: (Cont'd.)

The equipment previously used for analysis of CO_2 and C-14 in the breath of patients has been inadequate for the intended applications. To remedy this, a new digital-recording system was built for digitizing the output of the existing analogue-type recorders. The output is recorded on punched paper tape which serves as the input for further processing by the computer.

Analysis of the data obtained in the membrane transport experiments has largely been completed. The effects of a range of sugar concentrations on the diffusion rate as measured with tritiated water are being interpreted in terms of the driving forces present in the system.

No progress was made in the proposed rebuilding of the positron scanner, or in the development of the previously-proposed optico-electronic scanning system due to lack of the required financial support in these areas. (Robertson)

17. Expected Results in FY 1971:

A new thyrocalcitonin preparation is in the process of being purified by Armour. If approved by the FDA for clinical trials, its therapeutic effects on osteoporotic patients will be studied, using the experimental techniques described above. One study is planned to measure the rate of Ca loss from the body in normal aging. Another is planned to investigate disorders of the skeleton, other than osteoporosis.

Clinical research projects involving whole-body counting that will be continued include: measurement of levels of lean body mass (by means of K-40 measurements) in grossly obese children (the relation between total cell mass and fat cell mass should have basic physiological significance); studies on the kinetics of Br-82-halothane and other anesthetic gases; study of changes in the distribution of Mn^{++} following treatment of patients with Parkinson's disease with L-Dopa; and measurments of large and selected BNL populations on a routine basis. (Cohn)

Iron and vitamin B_{12} absorption tests will be standardized using the whole-body counter and its computer system. Then, patients with known pernicious anemia will be studied for turnover rates and redistribution of vitamin B_{12} within the body. The influence of anemia and the rate of erythropoiesis upon iron absorption and distribution will also be studied. The simultaneous absorption of vitamin B_{12} labeled with Co-57 and Co-60, when given with intrinsic factor, should provide a test for the immediate diagnosis of intrinsic factor deficiency or small bowel deficiency in the presence of macrocytic hyperchromic anemia. Studies are also planned on the role of small molecular weight components of lyophilized juice in respect to iron and vitamin B_{12} absorption. (Cronkite)

(See Continuation Sheet)

17. Expected Results in FY 1971: (Cont'd.)

A new study is in preparation (in collaboration with Dr. Letteri, Meadowbrook Hospital) which will provide analysis by means of in-vivo activation of Ca, Na, Cl and N in patients undergoing hemodialysis, and before and after kidney transplantation. Acute episodes of articular and periarticular inflammation occur in patients receiving periodic hemodialysis for chronic renal failure. These inflammations appear to develop in association with metastatic calcification, although serum Ca is normal. The effects of altering the concentration of elements in the perfusate on the Ca balance can readily be measured by the activation technique.
(Cohn, Cronkite)

The in-vitro neutron activation technique will be applied to sodium, chlorine and nitrogen, in addition to the present program with calcium. These measurements will be of direct value in the hemodialysis experiments; they should prove useful in lean-body mass studies, and possibly in hypertension studies. It is hoped that the portable 238 PuBe source facility in the Medical Research Center can be completed.

Work on the formulation and testing of mathematical models, particularly those for describing long-term retention data, will be curtailed until replacement appointments can be arranged. (Cohn)

F-18-labeled compounds, in addition to F-18 phenylalanine, should be available for visualization of bone, pancreas and perhaps adrenals. Hopefully, C-11-amino acids and L-Dopa will be available. Use of Fe-52 to investigate marrow distribution in leukemics will be studied.

Further clinical application of lymph node scanning is contemplated, particularly with improved colloids. With improved apparatus, extension of absorption edge spectroscopy will be extended to include clinical studies. (Atkins)

The imminent completion of the connection of the Sigma 2 computer to the Brooknet system will require a shift in the programming emphasis from further development of the whole-body counter and EPR systems to that of meeting the requirements for interfacing and for establishing communication dialogues between the Sigma 2 and the central computers. A variety of data processing services such as card to tape, paper tape to magnetic tape and various editing facilities will be available.

The increased convenience of computing batch-process jobs that will result from the Brooknet connection may be expected to stimulate computer usage in the Medical Department. The precise requirements for data processing assistance cannot be clearly predicted, but appreciable growth over the present level is anticipated.

Interest continues in the use of the multidetector positron scanner as a tool for investigating regional cerebral blood flow. However, the

(See Continuation Sheet)

17. Expected Results in FY 1971: (Cont'd.)

existing equipment is too slow and has too high a background count to be usable in some of the desired studies. It is again planned to modify the device by the addition of a PDP8L or similar small computer to serve principally as a data storage device. This and other changes in the circuitry would greatly enhance the machine's usefulness in blood flow studies. More extensive changes to improve the resolution and sensitivity for brain tumor localization are under consideration.

In addition to numerous modifications and improvements needed for the particle size distribution analyzer presently in use in the large animal facility, a complete duplicate system is planned for use with human blood samples in the clinical service laboratory. Experiments should be completed to determine the best geometry for the design and construction of a mouse activity system to be used with experiments involving the effect of L-Dopa on mice. This system would keep a record of a large number of mice showing general activity and some specific movements of each mouse as a function of time.

Investigation of the possible uses of laser holography in conjunction with radioisotopic scanning is planned. There appear to be very useful possibilities for image enhancement and 3-dimensional displays using holography. (Robertson)

18. Expected Results in FY 1972:

Studies will continue along the general lines described above but modified by findings suggesting new directions, and by the development of new techniques, such as neutron activation with (α,n) sources. (Cohn, Cronkite)

Further investigations with short-lived positron emitting nuclides are planned with particular interest in C-11-labeled amino acids, Dopa or derivatives, thymidine, etc. Nuclides may be available through the LINAC program of the Department of Applied Science. Feasibility of a ultra-short-lived generator system may be investigated. (Atkins)

The increased computer utilization by Medical Department personnel will necessitate increased supportive services. Long-range planning includes consideration of the development of a more independent medical computing facility with the addition of an analytical mathematician, a statistician, one or two programmers and a keypunch operator to the staff. In addition to enhancing the ability to handle the Department's data processing requirements, this would provide assistance in theoretical considerations of radiation effects or other appropriate topics. (Robertson)

(See Continuation Sheet)

Project Title: Exposure to External and Internal Radiation - Medical Research In-Vivo Measurement of Radionuclides in Man; Body Burden and Kinetic Factors. Computer Applications 06-02-01-(b)

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

A portable PuBe-238 source of fast neutrons for use within the Hospital area is an urgent need for these studies ($25,000). The facility provides the advantages of ease of operation (no full time operator required), ease of nonambulatory patient exposure (bilateral simultaneous with subject horizontal), and one-fifth the absorbed dose to the patient. This neutron source would also be very valuable in research, and would provide BNL with the finest in-vivo neutron activation facility in existence today. (Cohn)

A complete breath analyzer system for small animals, based on the method used for patients may be required. This would involve both the design and development of the CO_2 analyzer, a scintillation detector for C-14 and a digital readout system ($10,000). (Robertson, Shreeve, Hankes)

A small computer ($127,000) is required for quantitative assessment of distribution of activity in rectilinear scans and camera studies; multiple plane readout and stereoprojection of positron camera images; calculation of distribution of radioactivity with the 32-crystal positron device, calculations, corrections, etc. associated with the output of the transmission scans for absorption edge spectroscopy; time course of radioactivity distribution with the gamma camera-cardiac output, renograms, vascular flow studies; curve stripping and analysis for blood disappearance and liver uptake utilizing colloids; and subtraction of dual nuclide emission scans (FY 1971). (Atkins)

Other capital equipment needs include development of positron scanner, $20,000 (FY 1971); FY 1972, particle size analyzer, $15,000; and a Laser system, $5,000 (departmental use).

20. Proposed Obligations for Related Construction Projects, if any:

Bio-Med conjunct facility.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory
Laboratory

06-Biology & Medicine
Program

1. Contractor: Associated Universities, Inc.
Contract No.: AT-30-2-GEN-16
Task No.:

2. Project Title:
Combating Detrimental Effects of Radiation - Medical Research
The Role of Damage to Mammalian Hemotopoietic System in Death or Recovery of the Irradiated Mammal
189 No.: 06-237

3. Budget Activity No.: 06-02-02

4. Date Prepared: May 1970

PRIVACY ACT MATERIAL REMOVED

5. Method of Reporting:
BNL Annual Report
BNL Monthly Letter to AEC
Bulletin of the Medical Department

6. Working Location:
Brookhaven National Laborato:

7. Person in Charge:
E. P. Cronkite
V. P. Bond

Principal Investigator:
A. L. Carsten
C. V. Robinson
E. P. Cronkite

8. Project Term:
Continuing
From: To:

9. Man-Years:	FY 1970	FY 1971	FY 197
Sci., Res. Assoc. (Ph.D. or Equiv.)	1.0	1.0	1.0
Visiting Sci.	-	-	-
Prof. (B.S. or Equiv.)	-	-	-
Sci. & Eng. - Total	1.0	1.0	1.0
Technical	1.5	1.5	1.5
Adm. & Service	0.5	0.5	0.5
Guests & Research Collaborators	-	1.0	2.0
Total	3.0	4.0	5.0

10. Costs (In Thousands of Dollars):	FY 1970	FY 1971	FY 197
Labor (including benefits)	47	43	49
Mats., Trav., Dev. Subcont., Spec'l Proc.	4	4	7
Reactor, Accel., and/or Computer Usage	0	0	1
Allocated Technical Services	2	3	4
Gen. & Adm. Overhead			
Total Research Cost			
Research Division	75	70	85
Hospital Division	0	0	0
Research Costs	75	70	85

11. Reactor Concept:

12. Materials:

Reactor
Irradiation

06-237

1178823

PRIVACY ACT MATERIAL REMOVED

13. Publications:

Bond, V. P. and Sugahara, T. Radiosensitivity in Animals. Science 164, 1428-35 (1969).

Reincke, U. Methods to determine volumes of hematopoietic nodules in bone marrow-grafted irradiated mice. Exp. Hematol. (in press).

14. Scope:

The studies in this activity have as their primary objective the development of basic information on mammalian radiation injury that in turn will serve as a basis for improved therapeutic applications. In the investigation of relationships between radiation effects at the animal and cellular levels, specific areas of interest include: a) the acute hemopoietic syndrome; b) radiation-induced leukemia; c) effects of nonuniformity of dose distribution; d) effects of dose protraction on the width of the animal dose-survival curve and on the 50% lethal dose; and e) development of improved substitutive therapy (granulocyte and platelet transfusions).

In one area of these studies, mathematical models which simulate existing data are formulated as an approach to problems in which the response of a single cell type is critical. In such cases the model must take account not only of the primary radiation response of such cells, but of the proliferation which they initiate, and the animal's requirement for, or sensitivity to, progenies of the irradiated cells. Analyses are made using the theory of random variables and stochastic processes, Fourier transforms theory, computer simulation, and the theory of competing risks. Experimentation is designed for filling in gaps or clarifying discrepancies in existing data. As these interpretive studies are phased out additional emphasis will be placed on cellular studies and substitutive therapy. (Robinson)

A major aim is understanding the effects of radiation on the cellular renewal systems essential for survival after irradiation. Relationship between kinetics of hematopoietic cell proliferation and radiation effects takes two forms: first, the effect of uniform whole-body, partial-body or non-uniform exposures on the hematopoietic stem cell pool; and secondly, and conversely, the importance of changes in the stem cell population on the animal's reaction to a uniform whole-body, partial-body or non-uniform exposure. To study the latter the stem cell pool is modified by age, chemicals, preirradiation, etc. Short-term objectives include the perfection of techniques related to the harvesting, preparation and storage of marrow for study by each of several methods and the determination of the size and distribution of the active hematopoietic stem cell pool throughout the body. (Carsten)

New studies on platelet preservation and transfusion will be carried on in collaboration with the Blood Research Laboratory, U. S. Naval Hospital, Chelsea, Massachusetts. Radiation hemorrhage was shown years

(See Continuation Sheet)

14. Scope:

ago, to be due to thrombocytopenia which could be prevented by prophylactic transfusion of homologous platelets. Platelet transfusion controlled well established diffuse purpura but is relatively impractical since platelet viability is so short in vitro in all preservation media and techniques. The development of adequate platelet preservation techniques is essential for treatment of otherwise fatal radiation injury. The Blood Research Laboratory leads in the research on the preservation of red cells and, under the direction of Dr. Valeri, has developed an imaginative program on the in vitro evaluation of one platelet function that appears to be correlated with viability, namely, its volume regulation. The treatment of individuals with one tablet of aspirin removes the capability of platelets to agglutinate and thus shorten bleeding time. Platelet viability is studied in normal human volunteers by preparing and preserving platelets prior to the administration of aspirin and subsequent autotransfusion to see if the aspirin defects are improved by the transfusion of the pre-aspirin platelets. The ultimate test of platelet viability and function however, is the prevention or the treatment of thrombopenic bleeding. With the development of techniques to preserve platelets in a viable and functional state, the collaborative study will use the BNL model of the thoracic duct cannulated dog in whom the output of red cells in the thoracic duct is correlated with thrombopenia and platelet function. (Cronkite)

15. Relationship to Other Projects:

Lewis and Trobaugh, University of Illinois, Chicago, and Wolf and Trentin Baylor University, are interested in the factors determining the type of spleen colony arising from marrow injections into irradiated recipients. They are not, however, examining the total spectrum of colony type and size over the entire development period for both endogenous and exogenous colonies as done here. (Carsten)

Till and McCulloch, University of Toronto, developed the spleen colony technique and continue to use it in a variety of studies. The BNL study is a more quantitative approach in the use of the spleen colony technique, particularly where related to the time course development of the colonies and their differentiation. A most important difference between work here and elsewhere is the use of the BNL mouse, which is particularly suited to the spleen colony technique, i.e. it is much more efficient both in time of development and number of colonies formed than most other strains. (Robinson, Carsten)

As noted above, Valeri et al, Blood Research Laboratory, U. S. Naval Hospital, Chelsea, Massachusetts, are developing techniques for improved preservation of red cells, white cells, and platelets. (Cronkite)

16. Technical Progress in FY 1970:

The species screening studies for spleen colony growth in mice were completed for two rat strains, normal human, leukemic human, dog, guinea pig, cat, rabbit, hamsters, gerbils, and rhesus and spider monkeys. Marrow

(See Continuation Sheet)

16. Technical Progress in FY 1970: (Cont'd.)

from these donors was injected into recipient mice treated with both x rays and immunosuppressant drugs. A rise in colony count was found in all recipients, but cytological examination of individual cells indicated that in all except those mice receiving rat marrow, the colonies were endogenous to the mice. The rat to mouse technique was used to examine the effect of environmental temperature on marrow stem cells in irradiated and nonirradiated rats. It was found that protracted exposure of rats to a low environmental temperature (2°C) resulted in almost a two-fold increase in the number of colony forming units per femur. Following a dose of vinblastine, return of colony forming units (CFU) in the bone marrow to the pretreatment level was more rapid in the cold exposed rats than in those kept at 25°C.

The effect of storage media, time and temperature on the viability and type of resultant colony was studied for the mouse bone marrow. When the ratio of erythroid to total colonies is considered, it was found that in terms of relative depression in viability, erythroid colonies were depressed to a lesser extent than were the total colonies derived from cells kept for eight hours in saline G at either room or ice temperature. A lesser effect on this ratio change was noted for the cells kept in the CMRL 1066 tissue culture media. This study was completed for storage periods to eight hours.

The studies on the importance of distribution of the hematopoietic tissue to mortality following nonuniform exposures in the mouse were completed. The protection afforded by survival of hemopoietic stem cells in lesser exposed areas of the body is independent of anatomic site and dependent solely on the number of stem cells surviving. This is in verification of the preliminary results previously reported.

The marrow distribution data for the monkey (reported as complete last year) was verified with S. T. Taketa (Ames Research Center).

Changes in marrow stem cell numbers in male mice from 2-103 weeks of age was determined. The total marrow cellularity of both the male and female mouse femur increased rapidly until approximately 8 weeks of age, and then more slowly with increasing age. The total CFU content in the femurs of the same animals exhibited a similar early rise in both the males and females with a subsequent leveling off resulting in a decrease in the ratio of CFU to total cells with age. (Carsten)

The data on long term stem cell recovery following a maximum sublethal whole-body exposure (525 rads) to the BNL male mouse, was completed to 65 weeks post exposure. An immediate reduction in the marrow cellularity was evident, followed by a rapid return to near normal levels by 7-8 weeks post-irradiation. The femoral CFU content followed a similar pattern, however, the recovery was not quite as complete.

(See Continuation Sheet)

16. Technical Progress in FY 1970: (Cont'd.)

The studies of the growth and differentiation of the exogenous and endogenous spleen colonies in the BNL mouse were completed. A computer program was developed for determination of colony volumes. From a study of the rate of increase in the volume of red cell colonies it was concluded that they are monoclonal whereas granulocytic colonies appeared to be polyclonal. Volume doubling times are about 10 hours. (Carsten and U. Reincke, Research Collaborator in Residence, U. Freiburg, Germany)

Work was begun comparing the spleen colony technique and the implanted diffusion chamber technique for studying hematopoietic stem cells in the BNL mouse. Apparatus for the latter was assembled and the first chambers implanted. Preliminary results indicate that the technique developed by Boyum in Norwegian mice works equally well with the BNL strain. A strain comparison program (BNL, Ch 2 and AKR mice) was initiated in collaboration with A. Upton (State University of New York at Stony Brook) aimed at evaluating the hematopoietic stem cell systems in these strains. A laboratory was set up in the quarantine area for the handling of the outside strains and training of the personnel begun. (Carsten and A. Boyum, Norwegian Defense Research Establishment)

A series of studies aimed at determining the RBE of Californium-252 neutrons for the mouse marrow system was started. A source holder was fabricated to hold a Cf-252 source or tantalum array, and dosimetry completed on the system. (Carsten, Atkins)

17. Expected Results in FY 1971:

The hematopoietic stem cell studies using the diffusion chamber technique will be expanded. It is planned to determine the colony formation efficiency of the AKR mouse as compared to the BNL mouse, with special emphasis on the transplantation efficiency factor. Studies on the changes in marrow stem cell content with age in the BNL female mouse will be completed; also, determination of the RBE of the Californium neutrons for suppression of CFU's. It is expected also, that studies on the effects of storage of marrow for longer terms than previously reported, on mortality following nonuniform irradiation of mice and monkeys, and on the development and differentiation of exogenous and endogenous spleen colonies in the BNL mouse, will be completed.

Pilot studies will have started using the Luening embryo technique for studying the effects of various factors on the developing embryo. This system involves the breeding of male and female mice, one or both of which have undergone treatment previous to the mating (the treatment might be chemical, physical, etc.). The preganancy is allowed to continue until the 18th day, at which time the female is killed, and the number of viable and nonviable embryos counted, together with the number of corpora lutea on the ovaries. The importance of these values is self evident. The technique of counting the embryos has been confirmed and standardized. The enumeration of the corpora luea is not as easily accomplished, and at present various staining methods are being tested to

(See Continuation Sheet)

17. Expected Results in FY 1971: (Cont'd.)

improve this part of the system. It is planned that this method along with study of CFU's will be used to evaluate the toxicity of double-stranded RNA, L-Dopa, tritiated water, tritiated thymidine, and the relative toxicity of radioactive labeling in various positions in other biologically important compounds. (Carsten)

It is believed that studies on the evaluation of preserved canine platelets will have extended through the phase of showing that the aspirin defect can be reversed and studies on the evaluation in radiation-induced hemorrhage will commence. When improved techniques are established procedures will be proposed to Biological Control, NIH, for introduction into blood banking procedures. (Cronkite)

18. Expected Results in FY 1972:

With the data obtained by using the diffusion chamber technique and the spleen colony technique on several mouse strains, it is hoped to improve understanding of stem cell kinetics in the mouse. It is hoped also that new data on the relationship of labeling position in metabolites and radiation effect might be obtained with the Luening embryo technique. Studies will be continued based on findings during the previous fiscal year.

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

Continuous flow blood cell separation centrifuge ($60,000).

20. Proposed Obligations for Related Construction Projects, if any:

Bio-Med conjunct facility.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory — Laboratory

06-Biology & Medicine — Program

1. Contractor: Associated Universities, Inc.
Contract No.: AT-30-2-GEN-16
Task No.:

2. Project Title:
Cancer & Other Clinical Research--Medical Research
189 No.: 06-268

3. Budget Activity No.: 06-03-01

4. Date Prepared: May 1970

5. Method of Reporting: See sub-activities

6. Working Location: Brookhaven National Laboratory

PRIVACY ACT MATERIAL REMOVED

7. Person in Charge: See sub-activities

Principal Investigator: See sub-activities

8. Project Term: Continuing
From: To:

9. Man-Years:	FY 1970	FY 1971	FY 1972
Sci., Res. Assoc. (Ph.D. or Equiv.)	11.0	10.0	11.0
Visiting Sci.	4.0	4.5	5.0
Prof. (B.S. or Equiv.)	4.5	4.0	4.0
Sci. & Eng. - Total	19.5	18.5	20.0
Technical	79.5	70.0	73.5
Adm. & Service	9.5	9.0	8.5
Guests & Research Collaborators	4.5	2.0	3.0
Total	113.0	99.5	105.0

10. Costs (In Thousands of Dollars):	FY 1970	FY 1971	FY 1972
Labor (including benefits)	1,298	1,291	1,421
Matls., Trav., Dev. Subcont., Spec'l Proc.	244	240	257
Reactor, Accel., and/or Computer Usage	13	13	13
Allocated Technical Services	50	38	40
Gen. & Adm. Overhead			
Total Research Cost			

PRIVACY ACT MATERIAL REMOVED

11. Reactor Concept:

12. Materials:

SUMMARY

Sub-Activity	Title
	Medical Research
06-03-01-(a)	Treatment and Biochemical Dissection of Parkinsonism and Allied Conditions
06-03-01-(b)	Interrelationship between Genetic and Environmental Factors in Clinical and Experimental Hypertension
06-03-01-(c)	Clinical Studies on Exocrine Pancreatic Insufficiency, Vitamin B_{12} and Iron Absorption
06-03-01-(d)	Metabolic and Therapeutic Studies in Cancer
06-03-01-(e)	Extracorporeal Irradiation of Blood and Lymph in the Study of Lymphopoiesis and Homotransplantation
06-03-01-(f)	The Effects of Hormones, Drugs, and Nutritional Changes on the Intermediary Metabolism of Carbohydrates and Lipids

(See Continuation Sheet)

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory — Laboratory

06-Biology & Medicine — Program

1. Contractor: Associated Universities, Inc.
Contract No.: AT-30-2-GEN-16
Task No.:

2. Project Title:
Cancer and Other Clinical Research - Medical Research
Treatment and Biochemical Dissection of Parkinsonism and Allied Conditions
189 No.: 06-270

3. Budget Activity No.: 06-03-01-(a)

4. Date Prepared: May 1970

5. Method of Reporting:
BNL Annual Report
BNL Monthly Letter to the AEC
Bulletin of the Medical Department

6. Working Location:
Brookhaven National Laboratory

7. Person in Charge:
G. C. Cotzias

Principal Investigator:
G. C. Cotzias

8. Project Term:
Continuing
From: To:

9. Man-Years:

Direct Man-Years	FY 1970	FY 1971	FY 1972
Scientific & Professional	3.3	5.0	5.0
Other	35.5	27.2	26.3
Guests & Res. Collaborators	0.7	0.3	0.2
Total	39.5	32.5	31.5

10. Costs (In Thousands of Dollars):

	FY 1970	FY 1971	FY 1972
Research Division	298	360	368
Hospital Division	322	330	332
Research Costs	620	690	700

11. Reactor Concept:

12. Materials:

13. Publications:

Cotzias, G. C. Parkinsonism and dopa: an editorial. J. Chronic Diseases 22, 297-301 (1969). 1334

Mena, I., Horiuchi, K., Burke, K. and Cotzias, G. C. Chronic manganese poisoning: individual susceptibility and absorption of iron. Neurology 19, No. 10, 1000-5 (1969). 1345

Cotzias, G. C. L-dopa in Parkinson's syndrome. New Eng. J. Med. 281, 272 (1969). 13677

Mena, I., Court, J., Funzalida, S., Papavasiliou, P. S. and Cotzias, G.C. Modification of chronic manganese poisoning: treatment with L-dopa or 5-OH tryptophane. New Eng. J. Med. 282, 5-10 (1970). 13793

Cotzias, G. C., Papavasiliou, P. S., Fehling, C., Kaufman, B. and Mena, I. Similarities between neurological effects of L-dopa and apomorphine. New Eng. J. Med. 282, 31-3 (1970). 13781

Cotzias, G. C. Metabolic modification of some neurologic disorders. J. Am. Med. Assoc. 210, No. 7, 1255-62 (1969). 14033

Cotzias, G. C. Limiting factors in the treatment with dopa. Presented at the Laurentian Research Conference on L-Dopa, Val David, Quebec, Canada, November 1969. 14143

14. Scope:

Currently, the broad objective of the studies reported in this activity is the biochemical dissection of the defects in Parkinsonism and allied conditions in order to develop new approaches to therapy. Major efforts are still directed toward the study of the therapeutic effects of L-Dopa in the treatment of Parkinsonism, but an increasing effort is devoted to other chemicals and other diseases. A satisfactory standard of clinical investigation can be maintained, however, only with a relevant laboratory program tightly integrated with the work at bedside.

The therapeutic effectiveness of L-Dopa in the treatment of Parkinsonism has been firmly established. There remain, however, many questions as to how L-Dopa works. Explanations are sought for the following poorly understood observations: 1) large amounts of L-Dopa must be used for a considerable time before full improvement sets in; 2) the amount which controls symptoms must be lowered with passing months; 3) tremor can be eliminated simply by persevering with the maximum tolerated dose but other signs respond early; 4) some Parkinsonians treated with L-Dopa develop involuntary movements. Ascertaining which of the many active metabolites synthesized in the body from Dopa help the patient and which hinder, and predicting the degree and timing of improvement in a given case, also present related questions of interest.

(See Continuation Sheet)

14. Scope: (Cont'd.)

The brains of Parkinsonian patients contain low concentrations of both melanins and catecholamines. Both sets of missing substances can be restituted by their common precursor L-Dopa (3,4 dihydroxyphenylalanine). A premise for the studies in this budget activity is that the chemistry, not the structure, of the brain is responsible for Parkinsonian symptoms since they can be corrected with chemicals without correcting the underlying structural damage. Parkinsonian symptoms are found also in persons who have some other disease than Parkinson's syndrome. Accordingly, efforts are directed toward demonstrating that chemicals should improve such symptoms wherever they may occur, and not only in the case of Parkinson's syndrome.

Extensive laboratory studies are carried on to supplement, clarify, and prepare for clinical investigations. Body fluids of patients must be analyzed for L-Dopa and metabolites suspected of causing certain effects. For example, when a patient shows diminished effects of L-Dopa following a heavy meal, analyses must be made to determine whether the ingredients of the meal interfere with the absorption of dopa; when a patient shows increased plasma iodine, the nature and source of the iodine must be determined. Animal experiments must precede studies on human beings; rudimentary toxicological tests and analyses must be conducted on chemicals proposed to the FDA as investigational new drugs.

Carbon-11-L-Dopa is expected to render "visible" in the intact man (by means of technology already available) several extracranial and intracranial structures. Among the extracranial ones, the adrenal medulla promises to become readily visible but structures like the lung which are rich in dopamine-containing histiocytes may also become preferentially visible. The distribution of carbon-11-L-Dopa among extracranial structures is expected to be dependent not only upon the dose of non-radioactive Dopa but also on whatever metabolic adjustments have been brought on by its chronic administration. Studies on intracranial structures are even more important since visualization of structures located behind the skull is now limited to the few tumors which one may scan, the calcified pineal gland, the sella turcica, and the distortions that become evident by angiography.

An essential phase of the basic studies on neurological function is the continued development of nondestructive neutron activation analysis so that the inorganic analysis can be followed by organic analysis in the same sample. This capability is required in order to adequately analyze autopsy material and tiny biopsies of brain.

15. Relationship to Other Projects:

Investigations of L-Dopa have been spurred throughout the world and special conferences and international symposia have taken place or have been established. Work here is not addressed to problems if there is reason to suspect they are being formulated by others.

(See Continuation Sheet)

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

The synthesis of carbon-11-L-Dopa by Dr. A. Wolf and his associates in the Chemistry Department is a most important related work. Despite the fact that several outsiders have become interested in the studies on intracranial and extracranial structures, progress at Brookhaven has been slow due primarily to the difficulties in rapid synthesis and availability of adequate amounts of carbon 11.

Total body and regional counting is another intimately related project. The techniques and equipment developed at Brookhaven would be indeed applicable to eventual studies of C-11-L-Dopa but they are applicable here and now to studies of the effects of L-Dopa on trace metals and vice versa.

16. Technical Progress in FY 1970:

An article "Metabolic Modification of Some Neurologic Disorders", J. Am. Med. Assoc. 210, 1255-62 (1969) was invited by the Journal of the American Medical Association in connection with the author receiving the Albert Lasker Clinical Medical Research Award. The paper presented the background, current status and immediate prospects of metabolic therapy of neurological disorders. It indicated also, areas in which basic investigations are urgently needed.

In studies on functional interactions between biogenic amines, 3',5' cyclic adenosine monophosphate (cyclic AMP) and manganese, the relevant connection between the metabolism of Dopa and that of trace metals like manganese was shown. Others had discovered that biogenic amines do not deliver their intracellular messages directly but via an intracellular messenger, cyclic AMP.

Studies here determined that the increase of intracellular cyclic AMP caused by several amines including Dopamine is accompanied by a striking intracellular accumulation of manganese. This connection appeared important because it was known that one could mimic the effects of some amines by using cyclic AMP. One such effect was mimicked by administering manganese instead of either an amine or cyclic AMP. Efforts were devoted to the adaption of a method to permit measurement of cyclic AMP in tissues. Preliminary experiments suggest that administration of Dopa increases the concentration of cyclic AMP in the liver, the only tissue tested. Experiments are in progress to determine whether factors modifying the effects of Dopa change the concentration of cyclic AMP in liver. If so, these will be studied in brain, a more difficult organ to test.

A letter to the Editor, J. Am. Med. Assoc. 207, 1353 (1969), reported the emergence of positive Coombs tests among four Parkinsonian patients receiving large doses of L-Dopa for many months. The letter also stated that discontinuation of the drug was unnecessary and inadvisable because: 1) no evidence of hemolysis emerged; 2) the titers could be lowered by decreasing the dose of Dopa; 3) the patients needed Dopa quite badly.

(See Continuation Sheet)

16. Technical Progress in FY 1970: (Cont'd.)

Because of the large discrepancy between the negligible toxicity induced by L-Dopa in treatments here and the extensive toxicity encountered by some others, a letter was sent to the Editor of the New England Journal of Medicine to emphasize the difference between "dose" and "dose rate". A model schedule for administering Dopa was presented. Also indicated were precautions that must be taken and what support of the patient is recommended besides prescribing L-Dopa.

In studies on chronic manganese poisoning it was found that inhaled manganese compounds become largely transferred to the gastrointestinal tract. Contrary to prevailing opinion, therefore, the gastrointestinal tract is a major portal of entry during environmental and industrial exposure. Investigations of the absorption of manganese by this tract showed it to be linked to the absorption of iron. The percent of manganese absorbed (from that presented for absorption) depended upon the presence or absence of nutritional anemia. This suggested nutritional anemia as a predisposing factor to the development of chronic manganese poisoning among miners exposed to heavy manganese loads. An unexpected finding was that miners exposed to manganese were "iron losers". This iron loss would increase the percent of manganese absorbed by individuals to whom massive amounts of metal are already presented for absorption. These results could not have been obtained without the neutron activation analyses possible at Brookhaven.

The publication "Modification of Chronic Manganese Poisoning: Treatment with L-Dopa or 5-OH Tryptophane" by I. Mena, J. Court, S. Fuenzalida, P. S. Papavasiliou, and G. C. Cotzias, New Eng. J. Med. 282, 5-10 (1970), reports: 1) the first confirmation of the chemical hypothesis for the neurological symptoms in a disease other than Parkinson's; 2) the first successful treatment of a non-progressive, crippling neurologic disease; 3) the first results of administration of L-Dopa to neurologically normal human beings. In brief, the results indicate that seven out of eight long-crippled ex-miners benefited remarkably from the slowly increased administration of oral L-Dopa. The eighth, who became worse on L-Dopa improved with 5-OH Tryptophane, a precursor of serotonin. This latter case may have established a therapeutic precedent for similar clinical situations. Two of the miners who improved while on L-Dopa had primarily muscular dystonia, indicating that other dystonias must be similarly studied. Among eight normal individuals receiving L-Dopa, the six exposed manganese miners appeared to react differently than the two non-miners. The working manganese miners seemed resistant to the induction of nausea or vomiting by L-Dopa. They developed marked flexibility of their joints and diminished muscular tonus but no muscular weakness while on L-Dopa. The two normal controls differed from the exposed miners in these respects.

Another study was based on chemical similarities between dopamine and apomorphine and the fact that dopamine as well as apomorphine activate dopaminergic neurons. In this double blind study, 15 patients were

(See Continuation Sheet)

16. Technical Progress in FY 1970: (Cont'd.)

injected with 1.0-2.5 mg. of apomorphine or with placebo while their neurological status was scored intermittently. The scores changed only when apomorphine was injected. The change was in the same direction as when L-Dopa was given. It started upon injection, reached its peak after 30 minutes and declined to preinjection levels thereafter. It is proposed that apomorphine can be used to: 1) determine whether dopamine or melanins are responsible for the main therapeutic effects of Dopa; 2) to determine whether or not dopaminergic neurons become diurnally refractory in patients who lose the effects of L-Dopa following a meal or following ingestion of Vitamin B_6 (Pyridoxine); 3) as a model for the synthesis of a new generation of drugs with which to study and treat neurological diseases.

A test was developed which quantifies the locomotor effects of L-Dopa and their modification by various agents. This test is based on the fact that among mice receiving the same amounts of L-Dopa some become more hyperkinetic than others. A plastic box cage was constructed which has a three-inch platform running close to its top. Mice are placed on this platform. With placebo administration, no mice fall from the platform into the box, but with L-Dopa or apormophine, some do. The percentage of mice falling from the platform is calculated at the end of four hours. With mice of the same sex, age and body weight, the percentage of animals falling has been linearly related to the oral dose of L-Dopa between 1 and 5 mg/g body weight. Similarly, this percentage was linearly related to the dose of injected apomorphine. Previous administrations of dopamine or of 3,4 dihydroxyphenylacetic acid (dopac, a deaminated metabolite of dopamine) have markedly diminished the locomotor effects of L-Dopa but not those of apomorphine. The effects of both drugs were, however, delayed as well as diminished by the intraperitoneal administration of 40 μg of manganese as the sulfate or the chloride. These experiments may provide tools for selectively modifying the entrance of L-Dopa into the brain, versus the action of dopamine (or apomorphine) within the brain.

Connections between the metabolism of manganese and the administration of some agents utilized in therapy were sought in experimental animals. The effects of L-Dopa on manganese metabolism reflected themselves in the total body economy of this metal in mice as follows: injected Mn-54 was transported in the presence of Dopa much more rapidly from the carcass into the viscera in the presence versus the absence of this drug ($0.01 > p > 0.001$); the concentration of Mn-55 in the livers of mice as determined by neutron activation analysis became progressively higher with time on L-Dopa; among two peripherally acting Dopa-decarboxylase inhibitors, one markedly diminished the absorption of manganese from the gut in mice. This is the inhibitor used extensively in Europe, Latin America, and Canada. The one used at BNL has not yet shown evidence of interfering with the absorption of manganese.

Parkinsonian patients who have been receiving L-Dopa for more than three years were closely followed. In most, the initial improvement is maintained and no new toxicity has been found. One patient has developed

(See Continuation Sheet)

16. Technical Progress in FY 1970: (Cont'd.)

a gastrointestinal hemorrhage which is tentatively assigned to a hiatus hernia. A few patients have developed intermittent involuntary movements and variable degrees of intermittency in the control of their Parkinsonism. It seems that the absorption of Dopa is becoming uneven in some individuals. The role of the intestinal flora in this intermittency is being investigated by means of sulfathiazole administration.

Administration of α-methyl Dopa hydrazine (a peripheral Dopa decarboxylase inhibitor) together with L-Dopa is being studied to determine which effects of Dopa are caused by metabolic processes occurring outside the brain as well as to determine whether there are clinical advantages to this combination. Among seven patients fully studied thus far, the following trends have emerged: 1) the doses of oral Dopa necessary to control Parkinson's syndrome appear to be about one-fifth smaller in the presence of the inhibitor than in its absence; 2) administration of Pyridoxine cancels the effects of Dopa in the absence but not in the presence of the inhibitor; 3) bucco-facial involuntary movements have emerged much earlier and with much smaller doses of L-Dopa in the presence as compared to the absence of the inhibitor; 4) in the presence of the inhibitor, the urinary concentrations of Dopa or Dopamine could not be determined due to technical reasons; 5) the percent of Dopa excreted as homovanillic acid is much smaller in the presence than in the absence of the inhibitor.

Three patients with dystonia musculorum deformans, two patients with cerebral palsy, one patient with "postencephalitic syndrome" and one with hemiplegia have been under treatment with L-Dopa. One of the patients with dystonia musculorum deformans developed marked improvement and another moderate improvement but only after several months of constant therapy as in-patients. In the others, the results seem encouraging but it is too early to tell.

Other studies underway but not completed include: the effect of phenylalanine ingestion on the Parkinsonian state both prior to and during control with L-Dopa; the effects of choline and methionine on the control of Parkinsonism; and the toxicological study of L-Dopa in mice. The proposed method for "homing" an amineoxidase inhibitor preferentially into the brain was reported as ineffective by our industrial colleagues at Eli Lilly and Company. An alternate approach is being investigated according to our suggestions.

17. Expected Results in FY 1971:

With present level support, current studies will be carried on to completion and additional studies initiated as conditions permit. In a collaborative study with Dr. Cohn of this Department an effort will be made to determine whether L-Dopa, α-methyl Dopa hydrazine, apomorphine and 5-OH tryptophane derange the metabolism of trace metals like manganese in

17. Expected Results in FY 1971: (Cont'd.)

man. With additional support additional patients would be studied in efforts to improve existing therapy, and links between autoimmunity induced by Dopa and trace metal metabolism would be investigated.

Effort would be concentrated primarily on the synthesis of carbon-11 Dopa and on testing it on animals. Since the logistics of the cyclotron do not permit constant production of the short-lived carbon 11, these workers would be expected to synthesize and test one or more of the following compounds: a) precursors of a monoamine oxidase inhibitor; b) esters of L-Dopa; c) modifications of the apomorphine molecule; and d) non-absorbable decarboxylase inhibitor affecting only the intestinal flora. This work will be supervised jointly with Dr. A. Wolf of the Chemistry Department. In addition, analytical difficulties with the measurements of metabolites of Dopa (particularly in the presence of inhibitors) and in the setting up of new analytical methods would be attacked.

It is hoped to answer the question of why some individuals require much more Dopa than others. If a Dopa-sensitive and a Dopa-resistant strain of mice or rats can be bred a genetic basis for CNS sensitivity to Dopa would be demonstrated. These animals could be tested for cohort sensitivity to other dopaminergic and to serotonergic agents and for differences in their handling trace metals like manganese.

During the coming fiscal year it is expected that the Chilean group will perform experiments designed primarily to elucidate the environmental roles of manganese and to improve the treatment of chronic manganese poisoning.

The promised utilization of the nondestructive feature of neutron activation analysis has received little attention due to scarcity of personnel. As was stated last year, brain biopsies from humans could be subjected first to neutron bombardment and analyzed nondestructively for selected radio-nuclides using available Li-drifted germanium crystals at low temperatures. Upon completion of this analysis, the same sample could be analyzed for selected organic constituents. Demonstration of the limits of this approach requires persistent work.

18. Expected Results in FY 1972:

It is hoped it may be possible to proceed more rapidly toward better treatment for more diseases. The synthesis of carbon-11-L-Dopa and of related compounds should permit improving insight into the chemistry of the brain as it relates to function. The genetic separation of Dopa-sensitive from Dopa-resistant mice would provide opportunities to apply environmental means in changing the genetic substrate. Such environmental means could become possible therapeutic options.

Project Title: Cancer and Other Clinical Research - Medical Research Treatment and Biochemical Dissection of Parkinsonism and Allied Conditions 06-03-01-(a)

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

Primary requirements are for additional scientific and technical personnel to accomplish more laboratory and clinical studies than present support permits. There is ample current work for two additional physicians, an organic chemist , a chemically trained technician and a clerk. Capital equipment needs include $5,000 for automation of analyses of Dopa, Dopamine and its metabolites, and $15,000 for miscellaneous equipment. Between $50,000 and $100,000 for drugs and chemicals can be used.

20. Proposed Obligations for Related Construction Projects, if any:

Bio-Med conjunct facility.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory
Laboratory

06-Biology & Medicine
Program

1. Contractor:
Associated Universities, Inc.

Contract No.:
AT-30-2-GEN-16

Task No.:

2. Project Title:

Cancer and Other Clinical Research - Medical Research
Interrelationship between Genetic and Environmental Factors
in Clinical and Experimental Hypertension

189 No.:
06-279

3. Budget Activity No.:

06-03-01-(b)

4. Date Prepared:

May 1970

5. Method of Reporting:

BNL Annual Report
BNL Monthly Letter to AEC
Bulletin of the Medical Department

6. Working Location:
Brookhaven National Laboratory

7. Person in Charge:

L. K. Dahl

Principal Investigator:
L. K. Dahl
W. W. Shreeve
K. Knudsen

8. Project Term:

Continuing

From: To:

9. Man-Years:

Direct Man-Years	FY 1970	FY 1971	FY 1972
Scientific & Professional	3.0	3.0	4.0
Other	22.8	15.8	24.6
Guests & Res. Collaborators	0.2	0.2	0.4
Total	26.0	19.0	29.0

10. Costs (In Thousands of Dollars):

	FY 1970	FY 1971	FY 1972
Research Division	234	245	320
Hospital Division	211	175	320
Research Costs	445	420	640

11. Reactor Concept:

12. Materials:

Dahl 6
Shreeve 8
(Fri ??)

06-279

1178840

Cancer and Other Clinical Research - Medical Research

Project Title: Interrelationship between Genetic and Environmental Factors in Clinical and Experimental Hypertension 06-03-01-(b)

13. Publications:

Dahl, L. K. Salt and adrenals. Renal Hypertension, I. H. Page and J. W. McCubbin, Editors, pp. 264-74, Year Book Medical Publishers, Inc., Chicago, 1968.

Dahl, L. K., Knudsen, K. D. and Iwai, J. Humoral transmissions of hypertension: evidence from parabiosis. Circ. Res., Supplement I, 24 and 25, I-21-I-33 (1969).

Ben-Ishay, D. and Welner, A. Sensitivity to experimental hypertension and aggressive reactions in rats. Proc. Soc. Exp. Biol. Med. (in press).

Dahl, L. K., Heine, M., Leitl, G. and Tassinari, L. Hypertension and death from consumption of processed baby foods by rats. Proc. Soc. Exp. Biol. Med. (in press).

Knudsen, K. D., Iwai, J., Heine, M., Leitl, G. and Dahl, L. K. Genetic influence on the development of renoprival hypertension in parabiotic rats: evidence that a humoral hypertensinogenic factor is produced in kidney tissue of hypertension-prone rats. J. Exp. Med. 130, No. 6, 1353-65 (1969).

Iwai, J., Knudsen, K. D. and Dahl, L. K. Genetic influence on the renin-angiotensin system: evidence for a renin inhibitor in hypertension-prone rats. J. Exp. Med. (in press).

Jaffe, D., Sutherland, L. E., Barker, D. M. and Dahl, L. K. Effects of chronic excess salt ingestion: morphologic findings in kidneys of rats with differing genetic susceptibilities to hypertension. Arch. Pathol. (in press).

Barker, D. M., Sutherland, L. E., Jaffe, D. and Dahl, L. K. Effects of chronic excess salt ingestion: juxtaglomerular granulation in kidneys of rats with differing genetic susceptibilities to hypertension. Arch. Pathol. (in press).

14. Scope:

The major long-term objective in this activity is to understand the pathogenesis of hypertension so that more effective prevention and therapy for man can be devised. There is increasing clinical evidence compatible with a formulation developed here on the hypertensive process that the disease starts with the genetic substratum with which environmental influences interact to produce hypertension. The nature of these remediable environmental influences are studied on a relatively short-term basis. Many of these studies involve the two unique strains of rats developed at Brookhaven, one of which is predisposed to experimental hypertension (the S strain) and the other resistant to it (the R strain).

14. Scope: (Cont'd.)

Currently, a major effort seeks a genetic "marker" that will identify the hypertension-prone individual; both the genetic study and the renin-angiotensin investigations could provide clues to such a "marker". Since the time of Richard Bright, the kidneys have been invoked to "explain" hypertension and a study of its role is carried on here by transplanting kidneys from animals of one strain into nephrectomized members of the opposite strain. Aside from the unique genetic and experimental aspects, this study is unique also because essential hypertension is studied in contrast to Goldblatt hypertension--the latter having probably little relation to development of essential hypertension of man.

Studies are also carried on involving acute and chronic irradiation of the two strains of rats in order to ascertain the effectiveness of radiation as an environmental insult leading to the development of hypertension. Zeman and Carsten have observed that localized irradiation to the spinal cord of rats produced clinical and pathological manifestations of neurological injury much earlier in those animals with experimental hypertension than in normotensive controls.

There is also considerable interest in the proposal that the high salt content of baby foods could play a role in the development of hypertension in the adult and this is explored experimentally. Collaborative pathological studies are carried on with groups at the Hospital for Sick Children in Toronto, and at the New York University Medical School.

Clinical facts have been interpreted as indicating some metabolic interrelationships among hypertension, atherosclerosis, diabetes, gout, and obesity. Proof of such a relationship might have a unifying effect similar to that of the concept of the collagen diseases. Long-term studies are carried on to test this hypothesis; current studies of glucose tolerance, plasma insulin, and serum uric acid suggest that some non-diabetic hypertensives can act like diabetics and others like gouty subjects. These studies lead to exploring the possibility that nucleic acid metabolism is defective, and that the defects are interrelated biochemically, in these five disorders. As of FY 1972, the studies on intermediary metabolism of carbohydrates and lipids (06-03-01-f) will be consolidated into this budget activity.

15. Relationship to Other Projects:

Since the two strains of rats are unique, and not available elsewhere, there is no duplication of the work carried on here. There are, however, two other strains of rats now available in which the males, in particular, develop variable "sponatenous" hypertension. Various related studies are being carried out by the following: Smirk, et al at the University of Otago, Dunedin, New Zealand, on vascular reactivity; Aoki, et al at the University of Nagoya, Japan, on pathology and relation of hormones to hypertension; Sjoerdsma, et al at NHI, NIH, on catecholamine metabolism.

Cancer and Other Clinical Research - Medical Research
Interrelationship between Genetic and Environmental Factors
Project Title: in Clinical and Experimental Hypertension 06-03-01-(b)

16. Technical Progress in FY 1970:

The effects of several renal manipulations including uninephrectomy, unilateral renal artery constriction, and a combination of these two (Goldblatt procedure), were studied in two strains of rats with opposite constitutional predispositions to experimental hypertension. The protective value of intact renal tissue against hypertension was shown to be genetically determined. The Goldblatt procedure carried out on only one member of a parabiotic pair induced hypertension in this operated rat but significant hypertension developed in the intact partner only when the operated animal belonged to the strain predisposed to hypertension.

It was speculated that there were qualitative differences in the pressor signals of the two strains of rats. In the strain genetically predisposed to hypertension there are at least two pressor principles: (a) one which is common to both strains, not transmittable via the parabiosis junction and presumably related to the renin-angiotensin system; and (b) a second which is specific for the hypertension-prone strain and can be transmitted through the parabiosis junction. This transmittable agent is probably identical with the factor that produced salt hypertension and is associated with the salt-excreting mechanism. These results suggest that the hypertensinogenic factor, the effects of which were observed earlier (J. Exp. Med. 126: 687, 1967) comes from the kidneys of the hypertension-prone strain. The current work is a natural extension of that earlier report in which it was found that the reaction pattern to salt hypertension could be changed when a rat from each of the two strains was united in parabiosis, i.e., the normally hypertension-resistant animal not only developed significant hypertension, but did so prior to its hypertension-prone partner. The sum of these two reports tends to confirm the thesis that the basic mechanism in the different "kinds" of hypertension is probably identical but that different etiological factors may precipitate the development of hypertension.

In the continuing exploration of the origin of the hypertensinogenic factor detected by means of parabiosis between members of the two strains, a cardinal observation was that when renoprival hypertension developed in a nephrectomized parabiont from the hypertension-prone (S) strain, this elevation in pressure was not transmitted to its intact partner. These data were interpreted as indicating that a pressor agent from an intact S rat was absent in the nephrectomized S rat i.e. that the hypertensinogenic factor, produced by the S but not R rats, is made in kidney tissue.

In an earlier report on the behavioral differences in the two strains of rats it was observed that the S strain displayed less "inquisitiveness" when exposed to a standardized test than did the R strain. In the present study, members of the S strain were also consistently less "aggressive" than those of the R strain as manifested by their responses to thirst or pain. No conclusion is yet possible as to whether these behavioral differences play a role in the pathogenesis of hypertension.

16. Technical Progress in FY 1970: (Cont'd)

In the studies on genetic influence on the renin-angiotensin system, two kinds of analyses were carried out on intact members of both strains of rats that had been maintained on a low NaCl diet: 1) sensitivity to injections of angiotensin and renin, and 2) the influence of their respective plasmas on the reaction velocity of renin and its substrate in vitro. The pressor response of intact S rats on low NaCl diet was found to be significantly higher to angiotensin and lower to renin than that of similarly fed intact R rats. High NaCl intake was without effect on the pressor response of R animals; in some of the S groups, however, added dietary NaCl clearly increased the pressor response. In all S groups there was a tendency for high NaCl to increase the pressor response to angiotensin, although this was statistically significant ($p < 0.01$) only among the intact controls, those with a unilateral renal artery clip, and the uninephrectomized rats. The response to renin was increased by high NaCl in the control and uninephrectomized S groups only. In animals from the S strain only, bilateral nephrectomy influenced the pressor responses to renin and angiotensin in opposite directions; that to renin increased while that to angiotensin decreased. In vitro, plasma from hypertension-prone rats had less endogenous renin activity than plasma from hypertension-resistant rats. As expected, all plasma renin activity disappeared within 24 hours after bilateral nephrectomy. Plasma from intact R rats showed no inhibition. Twenty-four hours after bilateral nephrectomy, no inhibition could be demonstrated in plasma from either strain.

Operational interpretation of these findings suggests that controlling the reaction between renin and its substrate is an inhibitor that exists in the kidney and plasma of S rats but not in R rats. The relation between this inhibitor and the hypertensinogenic factor is still speculative but the fact that both appear to originate in the kidney of S rats suggests a possible connection or even identity. A schema was devised in which the renin inhibitor operates as part of the blood pressure-raising system rather than the opposite and also controls the renin activity level.

Studies on juxtaglomerular granulation in kidneys of rats with differing genetic susceptibilities to hypertension were continued. The concentration of renal renin was estimated by a histochemical technique involving an estimate of the number of renin granules per glomerulus (the "juxtaglomerular index", or JGI). Differences between the two strains (R and S) were present on the same salt intakes, and these were not related to differences in blood pressure. A step-wise decrease in the average JGI was found in the following sequence: R-low salt > S-low salt > R-high salt > S-high salt. These results are compatible with those reported for the renin-angiotensin studies: if the S strain has a renin inhibitor it would be reasonable to expect that renin concentrations in the kidney would be less. It is possible, for instance, that the renin

16. Technical Progress in FY 1970: (Cont'd)

inhibitor is effective at the site of production of renin, hence the lower JGI in S rats.

In a collaborative study on the effects of chronic excess salt ingestion, morphological findings from kidneys of R and S strain rats given diets of low or high NaCl content were studied. Without knowing strain or diet, observers rated the kidney changes as "normal", "minimal to mild" and "moderate to very severe". In all 16 R strain animals, the kidneys were rated as "normal" irrespective of NaCl intake; all 8 S rats on low NaCl received ratings in the second category "minimal to mild", and the remaining 8 were S rats on high NaCl, rated "moderate to severe". The changes in the S rats on low salt were qualitatively similar to the more severe lesions in the high salt S rats. The unique observation by the group at the Hospital for Sick Children, Toronto, concerned musculo-elastic pads, observed at sites of arteriolar branching; these were most conspicuous at the origins of small arterioles from larger arterioles in S rats. These pads appear to be the most vulnerable portion of arterioles to change and may cause obstruction to blood flow. Proliferative changes were present at these branch sites even among S rats on low NaCl - suggesting the possibility of low grade obstruction even without NaCl. It was speculated that salt might make them swollen, or enhance hyperplasia, or that the enchanced vasoconstrictor activity of arterioles in S rats might make the pads relatively more prominent in the constricted vessel. In any event, they might function as micro-Goldblatt clamps thereby initiating the vicious circle of rising blood pressure and increasing renal damage.

The experimental aspects of the study on genetic characterization of the heritability of blood pressure in two strains of rats with opposite predispositions to hypertension was completed. The heritability of blood pressure appears to be multigenic. The findings are consistent with the hypothesis that blood pressure regulation and the constitutional tendency to hypertensive disease depend on several autosomal genes; that in males the effects of these genes are hidden while in females the gene for lower blood pressure is dominant on one or more of the loci. Models with 2, 3, and 4 non-linked autosomal diallelic loci were tested. There is no unique solution; in general, the more complex models can be manipulated to correspond better with the observations. A complex model, however, should be justified by independent evidence. So many environmental factors influence the blood pressure that any purely genetic model which describes these particular data with reasonable success is as likely to be correct as one which reproduces them in great detail. The model selected, operationally, was the simplest model that gives a reasonably good prediction of the findings consisting of 2 loci, each with 2 alleles. With this simple model, there has been surprisingly good agreement with the human data against which it has been tested.

There is no other study of the heritability of experimental hypertension comparable with this one in numbers of animals, duration, and

16. Technical Progress in FY 1970: (Cont'd.)

elegance of design. (It hardly needs to be said that there could be no human study even remotely comparable: the genetic data simply do not exist.) Thus, this may be one of the most important studies in this activity for it sets the limits for the number of phenomena, critical for the development of hypertension, which must be identified.

Continuation of work on the role of the adrenal in the development of experimental hypertension centered on two areas: adrenal transplants, and steroidogenesis in vitro and in vivo. The former involves surgical adrenalectomy followed by transplantation of adrenocortical tissue from a member of the opposite strain. Only a preliminary feasibility study was carried out: 4 of 8 animals (2S and 2R) survived such transplants and a high NaCl diet. After more than 4 months on NaCl, the evidence suggests that there has been no fundamental change in the character of the blood pressure response. From this small series, therefore, the adrenals appear to play a supportive, not causative, role. The study has not been pursued more vigorously because of limited technical assistance.

The synthesis primarily of aldosterone, deoxycorticosterone, and corticosterone in isolated adrenals in vitro and in adrenal vein blood in vivo was studied in both strains of rats. Analyses are being carried out in the laboratory of Dr. John Rapp, Penrose Research Laboratory, Philadelphia Zoological Gardens. No evidence of a difference in synthesis of the 3 steroids cited above was found in the preliminary analyses. There is, however, suggestive evidence of a difference in adrenal function: in the conversion of deoxycorticosterone (DOC) to [18OH-DOC + corticosterone] more ends up as 18OH-DOC in the S rats. 18OH-DOC is a fairly recently described steroid with some hypertensinogenic properties. If these findings are confirmed, their implications would be very meaningful if the transplant study showed a reversal of salt susceptibility in the two strains. This was not the case, however, in the 4 transplant studies made so far, alluded to above.

A preliminary study of the phenomenon observed by Zeman and Carsten was initiated in some rats from the S strain. 41 adult rats were ranked in ascending order of blood pressure and assigned alternately to test and control groups. The test rats were exposed to 3500 rads of x irradiation in a single dose to the spinal cord from C6-T2. This study is still in progress but it is evident that much younger rats will have to be used in the future because of the high death rate among these older animals due to complications of hypertension and pulmonary infections unrelated to radiation effect. The four rats in the test group that developed hind limb paralysis were all from those with lower blood pressures; this may have been due only to the fact that such animals lived long enough to develop the syndrome rather than implying that lower blood pressures promoted the syndrome. A better assessment was initiated using young animals with equal pressures from both the S and R strains.

One study on the effects of chronic consumption of processed baby foods was completed. The 25 test animals all developed hypertension

(See Continuation Sheet)

16. Technical Progress in FY 1970: (Cont'd.)

(avg. 180-190 mm Hg) and 12 died during the 8 months of observation. This was in contrast to the 15 controls, none of which died and the BP averaged 141.3 mmHg at the end of the study. In a further study artificial baby foods are made here, to half of which NaCl is added in a concentration approximating that of the processed foods. This means that there will be but one variable in the diet which control and test animals receive, overcoming one objection to the previous studies in which the controls received low salt rat chow whereas the test animals received processed baby foods.

The study of disturbances of carbohydrate metabolism in human hypertension is based on the thesis that there is an interrelationship among sodium, hypertension, and carbohydrate metabolism. This concept was expanded to include the thesis that there is a metabolic interrelationship among hypertension, diabetes, gout, obesity, and atherosclerosis. Proof of such a relationship might have the same consequences as did the unification of the collagen diseases. It is pertinent to note that whichever of these 5 diseases is used as the index disease, the other 4 are more commonly found in association with it than in the population at large. As a group, hypertensives here have an insulin response to an oral glucose tolerance test like that of late-onset diabetics, i.e., an elevation in peak and duration of the plasma insulin response. After sodium restriction and fructose infusion significant elevation of plasma uric acid concentration has been found in most hypertensives. It is suspected that nucleic acid metabolism is defective in the 5 disorders mentioned since elevation in plasma uric acid is common to all of them.

17. Expected Results in FY 1971:

Investigation of the medullary system of the kidneys in the two strains of rats will be initiated to study the role of the interstitial cells of the renal medulla in the pathogenesis of experimental hypertension. The immediate goal will be to describe the histological appearance of the interstitial cells under different salt loads and correlate this with blood pressure and strain of rat. Subsequently the lipid substances present will be extracted, defined and their vasoactivity examined.

If selective inbreeding of a Japanese strain of rats develops a colony of "spontaneously" hypertensive rats comparative studies between the Japanese strain and the strains developed here will be undertaken. Selective inbreeding will be used also to develop substrains of the BNL rats using both strains with high and low plasma cholesterol. These substrains would be used in studies of the metabolic interrelationship between hypertension and atherosclerosis.

Studies on renal transplants between the two strains of rats will continue; also a series of studies of the renin-angiotensin system in rats. Continuation of several of the collaborative studies will be dependent on collaborative support. Collection of data on hospitalized patients will continue for studies on disturbances of carbohydrate metabolism in human hypertension.

(See Continuation Sheet)

17. Expected Results in FY 1971: (Cont'd)

It is hoped to complete the adrenal steroidogenesis study and initiate a study on adrenal transplant between strains. The current study of foods prepared here should be completed but may require re-checking.

18. Expected Results in FY 1972:

Studies will continue on genetic-environmental interactions in the pathogenesis of hypertension. Development of additional substrains of rats will continue.

The clinical study of metabolic interrelationships among hypertension, atherosclerosis, gout, diabetes and obesity will be pursued and expanded by the consolidation into this activity of the studies reported in 06-03-01-f. If adequate C-11 labeled compounds are available abnormalities in diabetes and obesity will be explored.

By including deuterated glucose (e.g., glucose-1-^{2}H or glucose-6-$^{3}H_2$) along with comparably labeled glucose-H-3, given to patients at the same time as glucose-C-14, it might be possible to evaluate hydrogen isotope effects in glucose metabolism by measuring conversion of tritium or deuterium to proximal metabolic products in which dilution would not be too high for deuterium measurement. Such products as glucuronic acid and ribose formed from glucose in the liver can be "trapped" by their complex with other agents which are excreted in the urine. This would aid interpretation of current metabolic studies with tritiated carbohydrates and also would be pertinent to questions about toxicity of tritium introduced into the biosphere by nuclear power reactors in the future.

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

None.

20. Proposed Obligations for Related Construction Projects, if any:

Bio-Med Conjunct Facility.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory
Laboratory

06-Biology & Medicine
Program

1. Contractor: Associated Universities, Inc.
Contract No.: AT-30-2-GEN-16
Task No.:

2. Project Title:
Cancer and Other Clinical Research - Medical Research
Clinical Studies on Exocrine Pancreatic Insufficiency, Vitamin B_{12} and Iron Absorption

189 No.: 06-288

3. Budget Activity No.: 06-03-01-(c)

4. Date Prepared: May 1970

5. Method of Reporting:
BNL Annual Report
BNL Monthly Letter to AEC
Bulletin of the Medical Department

6. Working Location:
Brookhaven National Laboratory

7. Person in Charge:
E. P. Cronkite

Principal Investigator:
E. P. Cronkite
S. H. Cohn
A. D. Chanana

8. Project Term:
From: To:
Project terminates June 30, 1970.

9. Man-Years:

Direct Man-Years	FY 1970	FY 1971	FY 1972
Scientific & Professional	1.5	-	-
Other	0.5	-	-
Guests & Res. Collaborators	-	-	-
Total	2.0	-	-

10. Costs (In Thousands of Dollars):

	FY 1970	FY 1971	FY 1972
Research Division	57	0	0
Hospital Division	23	0	0
Research Costs	80	0	0

11. Reactor Concept:

12. Materials:

Planning Office

06-288

1178849

Cancer and Other Clinical Research - Medical Research
Clinical Studies on Exocrine Pancreatic Insufficiency,
Project Title: Vitamin B_{12} and Iron Absorption 06-03-01-(c)

13. Publications:

Rubini, J.R., Matsui, K., and Rane, L. Blood proliferocytes in human and animal malaria. Acta Haematol. (in press).

14. Scope:

The primary objective of the major studies in this activity has been the development of improved therapy for individuals with chronic malabsorption and deficiency of iron and Vitamin B_{12}. Due to the lack of pancreatic enzymes, patients with chronic exocrine pancreatic insufficiency are unable to absorb fat and protein from their food which leads to chronic malnutrition and related complications. They have foul smelling bulky stools, painful disabilities, frequently become narcotic addicts, and require large quantities of the commercially available, unpalatable pancreatic extracts as replacement therapy. In the fast few years, Greene and Hirs of the BNL Biology Department, along with Clifford, Wass and Joel of the Medical Department have succeeded in preparing lyophilized bovine and porcine pancreatic juice estimated to be far superior to the commercial preparation of pancreatic juice in the treatment of diseases listed above. In the BNL preparation, Greene et al have found in addition to the recognizable pancreatic enzymes, several medium and low molecular weight fractions, whose presence and function, if any, have heretofore been unknown.

In experimentally induced pancreatic exocrine insufficiency in dogs, the effectiveness of the BNL preparation was compared with commercial preparations and the BNL preparation shown to be superior. In collaboration with the University of Kansas, clinical trials in human beings are planned.

Factors regulating the absorption of vitamin B_{12} and iron are still poorly understood. Vitamin B_{12} is an essential substance required in the metabolism of all cells of the body. Its deficiency produces pernicious anemia. Using radio-cobalt-labeled vitamin B_{12}, investigations of absorption, excretion, daily requirement, and whole-body turnover of vitamin B_{12} are made possible by the whole-body counter. Of particular interest, is the long-term metabolism of vitamin B_{12} and its redistribution in the various organs of the body.

Iron is part of the hemoglobin molecule carried by red blood cells and is vital to oxygen transport throughout the body. Iron metabolism, with particular reference to iron absorption and retention, is also of considerable interest. Iron deficiency and overabundance may be signaled by iron absorption tests using the whole body counter.

Abnormalities of iron metabolism and vitamin B_{12} absorption develop in chronic pancreatic insufficiency; therefore, these studies will be performed on patients prior to and during the course of therapy with the BNL lyophilized pancreatic preparation. Also, as the low molecular weight fractions of pancreatic juice become available, their influence on the absorption of vitamin B_{12} and iron may be studied in animals.

15. Relationship to Other Projects:

Collaborative studies on the pancreatic enzymes are carried on at the BNL Biology Department and with Dr. Arthur Klotz, University of Kansas Medical School.

Several institutions in the United States and abroad are studying iron and B_{12} metabolism; however, currently, none have the unique capabilities of the BNL whole-body counter for determining rapid changes in the distribution of the radioactive tracer in the body.

16. Technical Progress in FY 1970:

In the past 12 months pancreatic juice was collected from 8 pigs and 3 cows and stored for future clinical and animal trials. Clinical studies have recently been initiated by Dr. Klotz at the University of Kansas. Studies at BNL were postponed, pending the availability of a gastroenterologist.

The necessary interfacing of the whole-body counter with the computer system has been achieved for analysis of rapid changes in distribution and retention of labeled test substances. Efforts in quantitation of redistribution of vitamin B_{12} after oral ingestion were initiated. One patient newly diagnosed to have pernicious anemia was tested. However, further work on standardization of methodology with vitamin B_{12} and iron was not accomplished due to shifts in personnel.

17. Expected Progress in FY 1971:

Further studies in these areas cannot be carried out until budget permits employment of an additional clinical research physician. When studies are recommenced they will be reported in Budget Activity 06-02-01-(b).

18. Expected Results in FY 1972:

Completed

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

None

20. Proposed Obligations for Related Construction Projects, if any:

None

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory
Laboratory

06-Biology & Medicine
Program

1. Contractor: Associated Universities, Inc.
Contract No.: AT-30-2-GEN-16
Task No.:

2. Project Title:
Cancer and Other Clinical Research - Medical Research
Metabolic and Therapeutic Studies in Cancer
189 No.: 06-291

3. Budget Activity No.: 06-03-01-(d)

4. Date Prepared: May 1970

5. Method of Reporting:
BNL Annual Report
BNL Monthly Letter to AEC
Bulletin of the Medical Department

6. Working Location:
Brookhaven National Laboratory

7. Person in Charge:
J. S. Robertson
E. P. Cronkite

Principal Investigator:
H. L. Atkins J. L. Bateman
E. P. Cronkite A. D. Chanana
L. V. Hankes

8. Project Term:
Continuing

From: To:

9. Man-Years:

Direct Man-Years	FY 1970	FY 1971	FY 1972
Scientific & Professional	5.5	6.5	6.5
Other	15.5	13.8	15.5
Guests & Res. Collaborators	2.0	-	-
Total	23.0	20.3	22.0

10. Costs (In Thousands of Dollars):

	FY 1970	FY 1971	FY 1972
Research Division	360	414	423
Hospital Division	90	69	107
Research Costs	450	483	530

11. Reactor Concept:

12. Materials:

Atkins 3
Hankes 10
Bateman 29
Chanana 26

1178852

Project Title: Cancer and Other Clinical Research - Medical Research
Metabolic and Therapeutic Studies in Cancer 06-03-01-(d)

13. Publications:

Schiffer, L. M., Chanana, A. D., Cronkite, E. P., Greenberg, M. L., Rai, K. R., Stryckmans, P. A. and Vincent, P. C. L'irradiation extra-corporelle du sang dans la leucemie lymphoide chronique. Nouvelle Rev. Franc. Hematol. 8, No. 5, 691-700 (1968).

Fairchild, R. G., Drew, R. M. and Atkins, H. L. The relative biological effect of ^{252}Cf radiation on HeLa cells in culture. Radiology 93, No. 5, 1187-9 (1969).

Hankes, L. V., Brown, R. R., Leklem, J. E. and Schmaeler, M. Metabolism of D- and L-tryptophan-7a-C^{14} in rats and the effects of unlabeled enantiomers. Proc. Soc. Exp. Biol. Med. (in press).

Archambeau, J. O. The effect of increasing exposures of the $^{10}B(n)$ ^{7}Li on the skin of man. Radiology 94, 179-87 (1970).

Cronkite, E. P. Acute leukemia: is there a relationship between cell growth kinetics and response to chemotherapy. Proc. 6th National Cancer Conference, American Cancer Society, National Cancer Institute, Denver, Colorado, September 1968 (in press).

Hankes, L. V., Leklem, J. E., Brown, R. R., Mekel, R.C.P.M. Abnormal tryptophan metabolism in human pellagra. Metabolism (in press).

Fairchild, R.G., Drew, R. M. and Atkins, H. L. Dose rate effects for various dose rates of ^{252}Cf radiation on HeLa cells in culture. Radiology (in press).

Fairchild, R. G. Dosimetry of ^{252}Cf. Proc. Symp. on ^{252}Cf, New York City, October 1968, J. J. Barker, Editor, pp. 277-84, Division of Technical Information, AEC, Conf. No. 681032, 1969.

14. Scope:

The common objective in the various studies carried on in this budget activity is improved understanding, diagnosis and treatment of cancer. Metabolic processes in selected cancer patients are studied. Dosimetric studies are aimed at defining advantages of various radiation sources.

A primary interest is the evaluation of Californium-252 as a fission neutron source for interstitial radiotherapy. Among radiotherapists there is great interest in the use of fast neutrons for treatment of malignancies in order to circumvent the hypoxic protective effect. Because of the marked damge to normal tissue that can be produced with fast neutrons, however, extreme caution is used today in the evaluation of the radiobiological effects of neutrons; this is particularly true in the case of Californium 252 (Cf-252), since almost no previous experience with continuous, protracted neutron therapy exists. The plan to evaluate the effects of Cf-252 is to extend cellular level studies to normal tissues in animals and then to human tissues. Also, the physical character of the source is studied

14. Scope: (Cont'd.)

to gain experience in techniques which would make them more suitable for implant therapy. Clinical trials cannot yet be scheduled. (Atkins)

Extracorporeal irradiation of blood (ECIB) provides a method to deplete the body of radiosensitive cells that circulate in the blood e.g., the lymphocytes and leukemic cells, without exposing the body directly to radiation or to radiomimetic drugs. Current studies are designed to observe the behavior of various types of human leukemia in a long-term follow-up of patients through their periods of remissions and relapses. The clinical course of the disease is correlated with the status of proliferative activity of the leukemic cells, and the influence of conventional therapy both with and without ECIB is studied.

Cell kinetic studies involve either the injection intravenously of tritiated thymidine or of autologous lymphocytes labeled with tritiated cytidine. Samples of blood, bone marrow, and lymph nodes are obtained and processed for autoradiography (ARG). Tritiated thymidine is incorporated only by those cells which are in the stage of DNA synthesis. In a steady state equilibrium the generation time of a proliferative cell compartment which feeds cells into a nondividing compartment can be estimated from the labeling index of the nondividing compartment. By the rate of disappearance of tritiated-cytidine-labeled lymphocytes, estimates are made of the relative sizes of the two major populations of lymphocytes viz., one population which is rapidly exchanging with the blood compartment and the other which is relatively fixed in the tissues. These parameters are measured before and after ECIB. (Cronkite, Chanana)

The objective in another study is the labeling of the various tryptophan metabolites in different positions of their structure with carbon 14 in order to study their metabolic pathways in animals and in various human diseases. Tryptophan, an essential dietary amino acid, has been shown to produce ortho-amino-phenol-type compounds which can cause cancer in animal urinary bladders. The literature reports that in patients with various types of cancer and other diseases the levels of a number of tryptophan urinary metabolites are significantly increased. Thus the normal metabolic pathway of tryptophan metabolism in man is of interest.

Tryptophan metabolites such as anthranilic acid, kynurenine, hydroxykynurenine and hydroxyanthranilic acid have been labeled with carbon 14 and developed for the resolution of small quantities of the D- and L-isomers of kynurenine, hydroxykynurenine and tryptophan. The carbon-14 labeled tryptophan metabolites are studied in animals first and then in human beings with tumors, anemias, malaria, siderosis (scurvy) and pellagra. (Hankes)

The study of hematopoietic activity in patients with disseminated disease secondary to mammary carcinoma who are receiving semi-intensive therapy with conventional alkylating agents or antimetabolites provides a unique opportunity to assay these perturbations in several cell-renewal systems. Erythropoiesis is of particular interest in that the antagonism

(See Continuation Sheet)

14. Scope: (Cont'd.)

of androgen to cytotoxic agent may be studied and quantitated. Although the major considerations in this investigation are of basic and physiological nature, information of clinical value is a by-product. (Bateman)

15. Relationship to Other Projects:

Activities related to the Cf-252 work are the production and development program for sources for medical and other uses at the Savannah River Laboratory; also, determination of the physical parameters of Cf-252 emissions and evaluation of Cf-252 effects in small animals and tumor systems at M. D. Anderson Hospital. At present, only the former and BNL have Cf-252 for medical use, but this will change in the near future. It will be necessary for a number of institutions to collaborate in a clinical study for evaluation of therapeutic gains from Californium. (Atkins)

Related collaborative studies on kinetics of leukemic cell proliferation (with or without ECIB) are carried on by: S. A. Killmann, University of Copenhagen; H. Cottier, University of Bern; and P. Stryckmans, Institute Jules Bordet, Belgium. Other groups active in studies on cell proliferation or on effects of ECIB in human leukemia are Hayhoe and Field, England; Thomas, USPHS Hospital, Washington; and B. Barnes, Massachusetts General Hospital and Harvard Medical School. Efforts here will continue to provide leadership in kinetic studies of leukemia and treatment by ECIB without the risk of unnecessary duplication. (Cronkite, Chanana)

Since tryptophan metabolites labeled with carbon 14 in various positions of the benzene ring of the indole nucleus are generally unavailable due to difficulties encountered in their synthesis, studies with labeled compounds of this type are conducted only here. Related work in other laboratories includes studies by: C. Wagner at Vanderbilt University on tryptophan metabolite inhibition; Gholson at Oklahoma University on feedback control mechanisms; Lardy at Wisconsin on tryptophan metabolite inhibition of glycolysis; Altman at N. Y. Medical College on hormone effects on tryptophan pyrrolase; and Miller at University of Rochester on induction of the tryptophan pyrrolase enzymes. (Hankes)

16. Technical Progress in FY 1970:

In the studies on evaluation of Cf-252, cell cultures were irradiated at two dose rates and with an acute exposure to fission neutrons. At dose rates of 9 and 6 rads/hr, some cell division occurs but after correcting for this, the survival curves are identical to those obtained at 16 rads/hr and with acute exposures indicating no recovery of sublethal damage.

Irradiation under anoxic conditions was finally achieved. Correcting for the fact that no cellular division occurs during the period of anoxia, the oxygen enhancement ratio (OER) is 1.3.

Bean roots (Vicia faba) were irradiated in collaboration with Eric Hall of Columbia University. The OER in this system was 1.66. Pig skin irradiations were carried out with radium at 35 rads/hr and Californium at 12-13 rads/hr. Skin tolerance was established at 8400 rads with radium

(See Continuation Sheet)

16. Technical Progress in FY 1970: (Cont'd)

and 1725 rads with Californium, giving an RBE of 4.9. A computer program, developed for calculation of dose distributions with Californium and radium, yielded results very close to measured values. (Atkins)

Four patients with chronic lymphocytic leukemia (CLL) were studied following retransfusion of autologous lymphocytes labeled in vitro with tritiated cytidine, one patient before and after ECIB. ARG's on three are still in process, but results analyzed so far confirm earlier observations that there are two major populations of lymphocytes. One rapidly exchanges with the blood compartment and is 0.67 to 7.3 times the size of the circulating lymphocytic pool; the other is a slowly exchanging or relatively fixed population of cells in lymphoid tissues. ECIB depletes the cells in the circulating pool. Two patients in early stages of CLL were given ECIB for 1.5 and 4.5 months respectively with concomitant reduction in the total body lymphocyte mass. They are being followed for long term assessment of improved survival and delayed onset of complications of CLL.

A tritiated thymidine i.v. flash-labeling study was done in one patient with CLL and initial results of ARG's indicate a somewhat slow proliferation rate of lymphocytes as seen by labeling indices in blood and bone marrow. Two patients with chronic myeloid leukemia (CML) were similarly studied; ARG's of their bone marrow and blood are still under review. One patient with idiopathic granulocytosis was studied with tritiated diisopropylfluorophosphate (DFP)-labeled granulocytes. The ARG's of in vitro studies on this patient show that the labeling intensity of the cells in blood and bone marrow, as judged by mean grain counts over granulocytes, was equal. This indicates there is no overt difference in the cells in the two compartments as was suspected after earlier work. The slides from in vivo studies are still in process.

Analysis of data on DNA synthesis time of blood and marrow myelocytes was delayed for studies on a few more patients. The number of patients with CLL treated with ECIB (along with conventional therapy) now totals seventeen; six are alive and under follow-up. The data on these seventeen patients show a better survival than observed with conventional treatment in another thirty-four patients. The six patients with CML have shown variable response to ECIB. The median survival time for seventeen acute leukemia patients treated with ECIB was eight months, which compares favorably with conventional chemotherapy. No suitable patient with CLL was available during the past year for lymphocyte kinetic studies following extracorporeal irradiation of lymph. Technical preparations are in readiness for the first suitable patient. (Cronkite, Chanana)

In the tryptophan metabolite studies, the administration of L-tryptophan-7a-^{14}C to a rat after a load of 3-hydroxyanthranilic acid produced a component in the urine which contains the majority of the urinary activity. Further studies with other labeled tryptophan metabolites have now made it possible to bracket the part of the tryptophan metabolite pathway involving this unidentified component. Sufficient quantities of

(See Continuation Sheet)

16. Technical Progress in FY 1970: (Cont'd.)

this material must be accumulated in order to isolate and identify it.

In a study with carboxyl labeled 3-hydroxyanthranilic acid in man, 80% of the activity excreted in the urine appears as three main radioactive components with similar Rfs on autoradiographs of chromatographs of the urines. Sufficient quantitites of urine containing these components were not available for the isolation and identification of these components.

Work on the study of the metabolism of I-inositol-C-14 in pentosuric patients in South Africa continued with the labeled compounds involved in the metabolism of I-inositol to L-xyulose in man being isolated from the urine of a patient studied last year. A study of carbon-14 labeled tryptophan, kynurenine and hydroxykynurenine in patients was conducted at the F. W. Verwoord Hospital in Pretoria, South Africa. The patients were men active in the South African mining industries and with histories of silicosis and scleroderma. Blood and urine samples were brought back to Brookhaven for analysis. Urines were also obtained from South African patients with scurvy and pellagra.

The resolution of small quantities of the labeled tryptophan related compounds into the D- and L-isomers on six foot long - four inch diameter paper pulp columns was continued in order to provide material for animal and human studies. (Hankes)

In the studies on patients with disseminated disease secondary to mammary carcinoma, slit lamp biomicroscopic examinations of the optic lenses were performed periodically on the current patients. One patient recently deceased had an incidence of opacities far above normal for her age, after being treated with cytotoxic agents for over three years. A second patient, treated for three years, has twice the number of opacities compared to "normals" of her age. A third patient, treated for only eighteen months, has the anticipated incidence of opacities for her age.

Slit-lamp biomicroscopic examinations of mice who received a 30 day LD 75% dose of triethylene thiophosphoramide, one of the agents employed frequently in patients, showed substantially more opacities than control mice by eight weeks, and approximately the effect which would have been produced by 300-400 rads of 250 kVp x rays. A 30 day LD-25% dose of fluoruracil (5-FU) produced no detectable effect out to 18 weeks post treatment.

Clinical findings of note in the series of metastatic patients treated primarily with cytoxic agents include repeatedly confirmed objective improvement in 22 of 32 cases (69%). Survival of patients at consecutive yearly intervals after (0) the date of diagnosis of metastasis has been, for objective responders/nonresponders (0) 19/9, (1) 16/6, (2) 12/4, (3) 6/0, (4) 3/0, (5) 1/0. These results compare favorably with results of others reported in the literature. (Bateman)

(See Continuation Sheet)

17. Expected Results in FY 1971:

Studies on the evaluation of Californium 252 will include: further cell culture studies with Chinese hamster cells, additional pig skin irradiations with different dose rates, histological assessment of changes made, and long-term observations for late changes. Irradiation of dog bowel and bladder for evaluation of tolerance levels will be performed. Skin reactions at lower levels of irradiation may be carried out on human beings. (Atkins)

Previous work on chronic lymphocytic leukemia (CLL) indicated that ECIB might be of benefit to patients in the early stages of the disease. Efforts will continue to treat early cases with ECIB to reduce the total body lymphoid mass. These patients will then be followed for long periods to observe if their overall survival is longer than the patients treated by conventional methods. As suitable patients with CLL are available, treatment by depleting the lymphocytes by extracorporeal irradiation of thoracic duct lymph (ECIL) will be attempted in conjunction with lymphocyte kinetic studies prior to and following such treatment to observe if the population of cells influenced by ECIL is different from the ones depleted by ECIB. The therapeutic effectiveness of the two measures will be compared.

Recent work in Cambridge, England, has shown that in acute leukemia ECIB apparently causes a marked increase in the number of cells in active proliferation. The drugs which are most effective in cancer chemotherapy act by their ability to attack only the proliferating cells. The Cambridge results will be checked in patients here and if confirmed, these patients will be given chemotherapy immediately after ECIB with the hope of achieving improved therapeutic results. A similar collaborative study in childhood leukemia is being planned with Alvin Mauer, Children's Hospital, Cincinnati. (Cronkite, Chanana)

Urines collected from pellagra patients given loads of L-tryptophan or kynurenine and vitamin B_6 will be analyzed for kynurenine, acetylkynurenine, 3-hydroxykynurenine, o-amino-hippuric acid, anthranilic acid glucuronide, Indican, kynurenic acid, xanthurenic acid, quinolinic acid and nicotinic acid. The excretion of some components should be abnormal and the analyses may provide sufficient information to determine whether the abnormalities are the result of a stress-induced increase in the activity of tryptophan pyrrolase, a lack of feedback control of this enzyme by low tissue pyridine nucleotides, a concomitant deficiency of vitamin B_6, estrogen imbalance, or combinations of these factors known to alter tryptophan metabolism. Further progress is expected in the isolation of C-14 labeled components from the urines obtained from the pellagrins.

Study of the influence of metabolism of one isomer on the metabolism of the other in rats, guinea pigs, cats and human beings will continue and hopefully enhance the interpretation of data obtained with DL-tryptophan in animals and man.

17. Expected Results in FY 1971: (Cont'd.)

Work on the metabolism of I-inositol to L-xyulose in human beings should be completed. (Hankes)

Effort will continue to quantitate the antagonistic relation of cytotoxic agents to androgens for erythropoiesis, and to further document an apparent differential in erythrostimulatory effectiveness of fluoxymesterone from different sources. Slit-lamp biomicroscopic lens examinations will be conducted at more frequent intervals on the patients, to clarify the incidence of minute discrete subcapsular opacities observed with that expected for their age specific normal counterparts. (Bateman)

18. Expected Results in FY 1972:

Long-term evaluation of pig skin irradiation will be continued. Irradiation of metastatic lesions in lymph nodes and skin lesions will be carried out to develop techniques and to observe biological effects. Hopefully, clinical trials may be initiated with a collaborating institute. (Atkins)

The studies on leukemic cell proliferation and use of ECIB are expected to continue. Patients previously treated will be followed on a long-term basis; selection of patients for present and proposed studies and experimental therapy will depend upon the yearly reevaluation of results accumulated. (Cronkite, Chanana)

Study of tryptophan metabolism in scleroderma, scurvy, pellagra, malaria, anemias and other related diseases will continue. The isolation and eventual identification of a few of the unknown urinary tryptophan metabolites mentioned previously should provide critical information concerning the metabolism of this essential amino acid in disease states and determine the course of new studies. (Hankes)

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

Scanning equipment support described in Budget Activity 06-02-01-(b) is desired also for some studies planned in this activity. (Atkins)

20. Proposed Obligations for Related Construction Projects, if any:

Bio-Med conjunct facility.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory
Laboratory

06-Biology & Medicine
Program

1. Contractor: Associated Universities, Inc.
Contract No.: AT-30-2-GEN-16
Task No.:

2. Project Title:
Cancer and Other Clinical Research - Medical Research
Extracorporeal Irradiation of Blood and Lymph in the Study of Lymphopoiesis and Homotransplantation
189 No.: 06-299

3. Budget Activity No.: 06-03-01-(e)

4. Date Prepared: May 1970

5. Method of Reporting:
BNL Annual Report
BNL Monthly Letter to AEC
Bulletin of the Medical Department

6. Working Location:
Brookhaven National Laboratory

7. Person in Charge:
E. P. Cronkite

Principal Investigator:
E. P. Cronkite
A. D. Chanana
D. D. Joel

8. Project Term:
Continuing

From: To:

9. Man-Years:

Direct Man-Years	FY 1970	FY 1971	FY 1972
Scientific & Professional	4.2	2.8	4.5
Other	9.7	12.6	15.6
Guests & Res. Collaborators	1.6	1.5	2.4
Total	15.5	16.9	22.5

10. Costs (In Thousands of Dollars):

	FY 1970	FY 1971	FY 1972
Research Division	380	328	432
Hospital Division	45	52	88
Research Costs	425	380	520

11. Reactor Concept:

12. Materials:

Chemistry 24

Project Title: Extracorporeal Irradiation of Blood and Lymph in the Study of Lymphopoiesis and Homotransplantation 06-03-01-(e)

13. Publications:

Cronkite, E. P. The study of lymphopoiesis utilizing extracorporeal irradiation of the blood and lymph. Progress in Clinical Cancer, Irving Ariel, M. D., Editor, pp. 118-132, Grune & Stratton, New York, 1969.

Chanana, A. D., Cronkite, E. P., Joel, D. D., Sipe, C. R. Influence of thymectomy and extracorporeal irradiation of blood upon blood lymphocytes in goats. Presented at the 53rd Annual Meeting, Federation of American Societies for Experimental Biology, Atlantic City, New Jersey, April 1969.

Iorio, R. J., Chanana, A. D., Cronkite, E. P. and Joel, D. D. Studies on lymphocytes XVI: distribution of bovine thymic lymphocytes in the spleen and lymph nodes. Cell and Tissue Kinetics (in press).

Okuyama, S., Aronson, R. B., Chanana, A. D., Cronkite, E. P., Rai, K.R., and Schiffer, L. M. Attempts at isolation of lymphocytosis producing factor from supernatant fluids of bordetella pertussis cultures. Proc. Soc. Exp. Biol. Med. 133, 723-7 (1970).

Ruchti, C., Cottier, H., Cronkite, E. P., Jansen, C. R. and Rai, K. R. Studies on lymphocytes. XVII. differential lymphocyte depletion in lymphoreticular organs of the calf during continuous extracorporeal x-irradiation of the circulating blood. Cell and Tissue Kinetics (in press).

Cottier, H., Ruchti, C., Sordat, B. and Cronkite, E. P. Irradiation extra-corporelle du sang circulant: moyens d'etude des circulation et recirculation lymphocytaires chez le veau. Nouvelle Rev. Franc. Hematol. 8, No. 5, 679-84 (1968).

14. Scope:

There are two primary objectives in these studies: characterization of the kinetic model of lymphocytopoiesis leading to an understanding of the anomolous cell proliferation in certain human diseases and the development of methods useful in preventing rejection of allografts or in treating rejection episodes.

DNA and RNA labeling with tritiated purines and pyrimidines, and extracorporeal irradiation of blood (ECIB) and lymph (ECIL) in experimental animals are used in characterizing the kinetic model of lymphocytopoiesis. The labeling of lymphocytes can be carried out in the whole body, in single organs, or in vitro. Their subsequent behavior can then be followed within the animal or in the recipient after cross circulation of allogeneic or syngeneic labeled cells. These techniques are exploited in order to: estimate cell cycle times of various class of lymphocytes; study the influence of peripheral lymphocyte depletion (in blood and/or lymph) on cell cycle times and cell proliferation rates; measure cell production in and cell migration from individual lymphoid organs such as the bone marrow, spleen, and the thymus; characterize the mechanism of lymphocytosis produced by agents such as heparin and B. pertussis; and study the recirculating capacity of allogeneic and syngeneic lymphocytes.

14. Scope: (Cont'd.)

There is considerable evidence that the thymus regulates, at least in part, the size of the circulating pool of lymphocytes. The influence of thymectomy upon the sizes of the various lymphocyte pools utilizing isotope dilution and depletion methods (ECIB/ECIL) is studied. The inter-relationship between the thymus and the bone marrow, with particular reference to the interactions of bone marrow and thymus lymphocytes in immunological reactions, is investigated, utilizing the techniques of regional organ perfusion by radioactive isotopes. Similar techniques are used to study lymph node and splenic lymphocytopoiesis.

A continuing effort is made to develop a tissue culture chamber that is nourished extracorporeally by flowing blood and/or lymph. This system is designed to eliminate the need for synthetic culture media and thus provide a more physiological environment for the cell growth. Lymphocytes and HeLa cells will grow in this chamber when nourished by lymph alone. When perfected, the system is expected to offer opportunities for studying the differentiation of hematopoietic stem cells and the influence of humoral factors upon cell differentiation in immunological reactions. Work on an extension of such a culture system assembly incorporating extracorporeal oxygenation and perfusion has been initiated. This modified version of the ex-vivo culture system should be more suitable for clinical studies, in particular, the potentials of hemapoietic stem cells in various disease states such as aplastic anemias.

The ECIB and ECIL techniques to deplete the body of radiosensitive lymphocytes that circulate in the blood and lymph are used in conjunction with cell labeling techniques to study in detail the kinetics of lymphocyte production, migration, life span, and to acquire information on the regulation of normal lymphocytopoiesis.

Depletion of lymphocytes from the body leads to the possibility of testing whether this depletion is useful in suppressing immunological responses to allografted tissues and organs. Regional ECIL has afforded an opportunity of direct study of the influence of ECIL on immunologically activated lymphocytes and to trace the cellular pathways during immunological reactions to skin allografts. Prolonged repetitive ECIB can produce profound and sustained lymphocytopenia, prior to and following allotransplantation. From the results obtained with skin and kidney allografts, it appears that the level of blood lymphocytopenia is not the only important factor in prevention of allograft rejection. The daily ECIB sessions were restricted to a time required to radiate blood equivalent to five blood volumes because of the danger of exessive radiation damage to erythrocytes. In order to bring more lymphocytes under the influence of ECIB, longer daily sessions are desirable. This necessitates a reduction in the transit dose. Thus studies with low transit dose ECIB and renal transplantation in goats have been initiated.

14. Scope: (Cont'd.)

Effectiveness of ECIB and ECIL would be greatly enhanced for the purpose of immunosuppression and the therapy of lymphocytic leukemia were it possible to mobilize a greater than normal number of tissue lymphocytes into the blood stream. Studies to understand the mechanism of action of B. pertussis induced lymphocytosis are continuing in calves and goats. The toxicity associated with B. pertussis precludes its clinical use at this time. Thus, work continues on the separation of nontoxic lymphocytosis promoting factor from B. pertussis, utilizing techniques of column chromatography and using mice for the bioassay of various fractions obtained. Study of the regulation of lymphocytopoiesis is pursued with the development of techniques to study the proliferation within each lymphoid organ, the interchange between organs, and the size of lymphocyte pools.

15. Relationship to Other Projects:

Studies on cell proliferation utilizing tritiated pyrimidines were initiated at Brookhaven National Laboratory. Some of the units with special interest in this type of work are: The University of Ulm (Germany), Royal Cancer Institute (England), National Institutes of Health (U.S.A.), Jules Bordet Institute (Belgium), The University of Bern (Switzerland), the University of Copenhagen (Denmark), the University of California (Los Angeles and San Francisco). Extracorporeal irradiation of the blood and lymph, also developed at Brookhaven, is now carried on at the University of Washington (Seattle), Peter Bent Brigham Hospital (Boston), Massachusetts General Hospital (Boston), Medical College of Virginia, The University of Copenhagen (Denmark), The University of California (Los Angeles), French Atomic Energy Establishment (Grenoble), South African Atomic Energy Board (Pretoria), Royal Cancer Hospital (Surrey, England), Holt Radium Institute (Manchester, U.K.), Addenbrooks Hospital (Cambridge, England), The University of Göteberg(Sweden). No other unit is known to be engaged in the long-term intra-arterial perfusion of single organs with radioactive isotopes or in the study of the combined effect of ECIB/ECIL and lymphocytosis producing factors.

16. Technical Progress in FY 1970:

Renal transplantation was carried out in 30 goats. Control recipients survived from 15 to 18 days. The survival time of goats receiving high transit dose, pretransplant ECIB, ranged from 14 to 24 days. When low transit dose ECIB was initiated, the survival time in 3 goats receiving low transit dose ECIB (40-50 rads) ranged from 25 to 55 days. These early results are encouraging and more studies with various schedules of low transit dose ECIB are planned. Three lines of evidence urge further trials of ECIB in renal transplantation: (1) results with regional ECIL show the capability of this procedure to inactivate specific lymphocytes involved in allograft rejection; (2) results of the group at University of Copenhagen, Denmark, show effectiveness of ECIB as an adjunct to standard immunosuppressive therapy--(this group has shown that ECIB markedly reduces rejection episodes in human renal allotransplant recipients when there is good donor-recipient histocompatibility matching); (3) as mentioned above, initial

16. Technical Progress in FY 1970: (Cont'd.)

results here with low transit dose ECIB in goat renal allotransplants showed extended survival time.

Lymphocytotoxins were measured in 28 goats following renal allotransplantation. Some of these goats received ECIB or ECIB and 6 MP. So far there has been no significant correlation detected between the renal allotransplant rejection time and the appearance of lymphocytotoxins in these goats.

During the past year several experiments were carried out with an ex-vivo chamber. Technical features were modified to permit the chamber to serve as a reliable culture environment. One of the chief objects in the current series has been to establish the usefulness of the system for studies concerned with cell interaction in circumstances mimicking those in transplantation studies. HeLa was chosen as a convenient target cell to pose an immunological challenge to lymphocytes in culture. Characteristics of HeLa cell growth were followed for 40 to 100 hours. Additionally the mutual response of HeLa and calf thoracic duct lymphocytes grown within the same chamber space were observed. After 96 hours, the last 48 of which were spent in the presence of host lymphocytes, HeLa population is vigorous though showing patches of cell destruction not seen in the control chambers (HeLa not exposed to lymphocytes).

Additional information about the influence of ECIB on lymphoreticular tissues was obtained by planimetric and test point analysis. The differential loss of lymphocytes from separate area of the spleen, lymph nodes and the thymus of calves, as compared to that from blood and the thoracic duct lymph was analyzed. The degree of depletion with time during continuous ECIB, followed an exponential function with at least two components. These two components are thought to reflect the easily mobilizable and less easily mobilizable pools of tissue lymphocytes. Elimination of easily mobilizable pool of lymphocytes by continuous ECIB from all tissues studied was observed within 10 to 15 hours, indicating that the rate of exchange with blood is similar for this group of cells in various lymphoreticular tissues. The ratio of readily mobilizable to less readily mobilizable pools of lymphocytes, however, varies in different organs. Fifteen goats were utilized to study the influence of ECIB or ECIB/Pertussis on blood and tissue lymphocyte depletion. Five goats received ECIB alone and ten goats received ECIB and Pertussis. Preliminary analysis of the data suggests a greater lymphoid tissue depletion in the combination treated animals.

Calves with thoracic duct cannulation or single node efferent lymphatic cannulation were used to study the mode of action of the lymphocytosis promoting factor of pertussis. In conjunction with these studies, in vitro labeling of lymphocytes was carried out to detect any concomitant change in the cell cycle time. The results suggest that the

16. Technical Progress in FY 1970: (Cont'd.)

early phase of blood lymphocytosis is due to an increased mobilization of lymphocytes from the lymphoid organs followed by an inhibition of recirculation of blood lymphocytes which helps to sustain the lymphocytosis.

A mouse bioassay system was used in attempts to isolate a nontoxic lymphocytosis promoting factor (LPF) from B. Pertussis. A partial success was achieved in separating LPF from the histamine sensitizing factor, the hypoglycemia inducing factor and the lethal factor. Using a combination of Sephadex G 10 and Bio Gel P 100 column chromatography, a fraction was obtained which retained its LPF but lost lethal and hypoglycemia inducing factors and partially lost the histamine sensitizing factor. Results seem to confirm the hypothesis of Bergman and Munoz that histamine sensitivity following pertussis is not due to hypoglycemia per se. In contrast to the findings of Morse and Bray, results here clearly showed a dose response effect. Kalpaktsoglou et al have suggested that B. pertussis mobilized thymic lymphocytes and findings here of thymic weight loss with peripheral lymphocytosis are compatible with such a hypothesis.

During the last year 18 additional calves were utilized to study the lymphocytopoiesis in lymph nodes, the thymus and the spleen. The technique of long-term intra-arterial radioisotope infusion was employed. Eight calves received intra-arterial thymic perfusion, three calves received i.v. perfusion, and six calves received intra-arterial lymph node perfusion. Recently one calf has been studied with splenic infusion. Analyses of radioautographs are incomplete but the following information was obtained from these studies: (1) whereas newly synthesized thymic lymphocytes migrate via the veins and efferent lymphatics, the newly synthesized lymph node lymphocytes migrate via the efferent lymphatics and not via the veins; (2) the highest numbers of the thymic migrants are seen in the paracortical areas of the lymph nodes and the loose white pulp of the spleen; (3) the thymic migrants are also seen in the "non-thymic dependent areas" of the lymph nodes and the spleen; (4) thymus migrants are not seen in the bone marrow; (5) since thymic migrants are seen in the thoracic duct lymph, these cells must have recirculating capability.

Studies with thymus specific antigen, utilizing the techniques of indirect immunofluorescence confirmed the migration of thymic lymphocytes via the veins and lymphatics. Within the thymus, the expression of thymus specific antigen is inversely related to the cell diameter and the expression of histocompatibility antigen. These results suggest that the differentiation of thymic lymphocytes from a thymic state to a peripheral state occurs in a very brief period immediately before or after they leave the thymus.

Studies on the relationship of the thymus to lymphocyte proliferation and transplantation immunology were continued. The estimated half-time for the disappearance of small lymphocytes from the blood of thymectomized goats was 60 weeks. ECIB induced a greater and more

16. Technical Progress in FY 1970: (Cont'd.)

pronounced lymphocytopenia in thymectomized goats than in control anima. Surgical procedures (thymectomy, sham thymectomy, splenectomy and thymec sham splenectomy and sham thymectomy), were carried out at the age of 12 weeks. Follow-up has included blood counts, weight gain, blood lymphocyt sizing, in vitro response to PHA of blood lymphocytes, lymph node biopsies and general clinical condition; some of the goats have been followed up to 64 weeks. Excepting for some depression in the blood lymphocyte levels in thymectomized goats, no other clinically deleterious effects of thymectomy have been observed so far.

Recirculating capabilities of allogeneic lymphocytes were investigated in two sets of experiments in calves. Lymphocytes were labeled with tritiated thymidine. The results of the first experiment indicate that the allogeneic lymphocytes cannot recirculate in a previously sensitized, specific recipient.

During the past year, while carrying out ECIB in goats, it became obvious that the goat erythrocytes were more radiosensitive than human or bovine erythrocytes. A thorough investigation of human and bovine erythrocyte radiosensitivity had been done in the earlier years to plan the ECIB schedules. It now became necessary to look into the radiosensitivity of goat erythrocytes for better planning of ECIB schedules. Preliminary studies have been completed in six goats. Cr-51 tagged RBC's, which had been irradiated in vitro were used. Goat RBC's were exposed to 20,000, 50,000, or 75,000 rads. Preliminary results of this investigation reveal that both the osmotic fragility and red cell survival are reduced at lower dose of radiation as compared to bovine and human erythrocytes.

The collaborative study with the Meadowbrook Hospital concerning the role of ECIB in human transplantation was not initiated because of delays in opening the human transplantation unit at Meadowbrook.

One study with continuous centrifugation of calf thoracic duct lymph was carried out. The blood and lymph lymphocyte depletion was similar to that achieved by continuous ECIL in calves. Further studies were curtailed due to lack of technical help.

17. Expected Results in FY 1971:

Studies with low transit dose ECIB in goat renal transplantation will be extended and hopefully completed.

Definitive studies will be commenced on those goats that were thymectomized during the last three years. Collaborative work with Meadowbrook Hospital dependent upon the establishment of their renal transplantation unit should be initiated. A renal hemodialysis unit has now been functioning at that hospital for about two years.

17. Expected Results in FY 1971: (Cont'd.)

Studies on the radiosensitivity of goat erythrocytes and on the influence of combined therapy with ECIB and pertussin will be completed.

Ten liters of pertussin supernatant are being supplied by courtesy of Eli Lilly Company. Thus, attempts to separate a clinically useful, nontoxic lymphocytosis producing factor will continue; mice will be used for bioassay.

More definitive experiments are planned for use of the ex-vivo culture system. It is hoped that the in-vitro culture chamber incorporating the extracorporeal oxygenation system will be ready for preliminary trials. Three experiments concerning the lymphocyte pools and the recirculating capacity of allogeneic and syngeneic lymphocytes should be completed.

It is hoped to concentrate on the following aspects of thymic lymphopoiesis: duration of life of the thymic migrants, and migration of thymic migrants to Peyer's patch. It is hoped also that further work will throw definitive light on the absence or presence of "direct entry" lymph node lymphocytes. It is planned to extend studies on regional perfusion of spleen in calves and these studies will be supplemented by investigations into the marrow as part of the overall emphasis upon characterization of the kinetic patterns of lymphocytes.

Trials of a beta irradiator to define the potential usefulness of this machine for clinical purposes should be completed. Calf ECIL and goat ECIB will continue in efforts to work out a better method for the dose calculation with beta irradiators.

Further work on continuous centrifugation of the thoracic duct lymph will depend upon the availability of scientific and technical support.

18. Expected Results in FY 1972:

Studies should continue based upon the findings obtained in the previous year. The studies on thymic and lymph node lymphocyte production and migratory patterns will be supplemented by the investigations into the bone marrow and splenic lymphocytes.

A major activity is expected to be the work in ex-vivo culture chamber and in-vitro culture chamber with extracorporeal oxygenation for studying the immunological reactions and the reactivity of hemopoietic cells.

Definitive studies on thymectomized goats are planned for completion. It is hoped that most of the work on the attempted isolation of nontoxic lymphocytosis producing factor from B. pertussis also will be completed.

18. Expected Results in FY 1972: (Cont'd.)

Depending upon the level of scientific and technical help available it is hoped to start studies on the concentration of lymphocytotoxins and the role of these humoral antibodies in immunological enhancement. In order to better understand the role of single lymph node in immunological responses, it is expected to initiate studies with horseradish peroxidase as an antigen.

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

It is anticipated that development of more sophisticated ex vivo and in vitro culture systems will cost approximately $20,000 in FY 1971 and $10,000 in FY 1972. Purchase of two beta irradiators ($8,000) is also planned.

20. Proposed Obligations for Related Construction Projects, if any:

Bio-Med conjunct facility.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS

Brookhaven National Laboratory	06-Biology & Medicine
Laboratory	Program

1. Contractor: Associated Universities, Inc. | Contract No.: AT-30-2-GEN-16 | Task No.:

2. Project Title: Cancer and Other Clinical Research - Medical Research
The Effects of Hormones, Drugs, and Nutritional Changes on the Intermediary Metabolism of Carbohydrates and Lipids

189 No.: 06-308

3. Budget Activity No.: 06-03-01-(f)

4. Date Prepared: May 1970

5. Method of Reporting:
BNL Annual Report
BNL Monthly Letter to AEC
Bulletin of the Medical Department

6. Working Location:
Brookhaven National Laboratory

7. Person in Charge:
W. W. Shreeve

Principal Investigator:
W. W. Shreeve

8. Project Term:
From: To:
Effort will be consolidated with studies in 06-03-01-(b) in FY 1972.

9. Man-Years:

Direct Man-Years	FY 1970	FY 1971	FY 1972
Scientific & Professional	2.0	1.2	-
Other	5.0	9.6	-
Guests & Res. Collaborators	-	-	-
Total	7.0	10.8	-

10. Costs (In Thousands of Dollars):

	FY 1970	FY 1971	FY 1972
Research Division	120	78	0
Hospital Division	65	142	0
Research Costs	185	220	0

11. Reactor Concept:

12. Materials:

1178869

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Project Title: Intermediary Metabolism of Carbohydrates and Lipids 06-03-01-(f)

13. Publications:

Hoshi, M. and Shreeve, W. W. Chronic effects of mannoheptulose in hyperglycaemic-obese mice. Metabolism 18, No. 5, 422-6 (1969).

Lamdin, E., Shreeve, W. W., Slavinski, R. H. and Oji, N. Biosynthesis of fatty acids in obese mice in vivo. II. Studies with DL-Malate-2-^{3}H-^{14}C, Succinate-2,3-^{3}H-2,3-^{14}C, and DL-Isocitrate-2-^{3}H-5,6-^{14}C. Biochemistry, 8, 3325-31 (1969).

Hostetler, K. Y.,Williams, H. R., Shreeve, W. W. and Landau, B. R. Conversion of specifically ^{14}C-labeled lactate and pyruvate to glucose in man. J. Biol. Chem. 244, No. 8, 2075-7 (1969).

14. Scope:

The studies in this activity have as their broad objective, the better understanding of the interconversion and metabolic pathways of carbohydrates and fats. To this end, investigations are carried on in experimental animals and in human beings. Currently, emphasis is placed on diagnosis of early or pre-diabetic states in human beings by testing rates of oxidation of glucose-1-C-14 and galactose-1-C-14 when administered orally, as in clinical carbohydrate tolerance tests. Also, the differences among glucose, fructose, and sucrose in their lipogenic potential is under investigation with carbon-14-labeled sugars in normal rats preliminary to studies in human beings. These studies help to clarify mechanisms of susceptibility to hyperlipemia and hyperinsulinism as affected by different diets. Since certain oral contraceptive steroids also predispose to abnormal fat and carbohydrate metabolism, the interaction of dietary changes with different contraceptive regimens is of interest and studied in some obese or hyperlipemic patients. Abnormalities of insulin production in vivo and in vitro (isolated pancreatic islet) are examined and correlated with disturbances of fat and carbohydrate metabolism.

The metabolic pathways and rates of utilization and interconversion of carbohydrates and fats are studied mainly with the aid of the radioactive tracers, carbon 14 (C-14) and tritium (H-3). Various abnormalities in endocrine disease and upon manipulation of hormonal influences in human beings and animals are investigated. Diabetes, obesity, acromegaly, and hyperadrenal cortical function are the conditions of particular interest.

In all of these conditions the question of abnormal increase of hepatic gluconeogenesis is explored with the aid of C-14 and H-3-labeled precursors (lactate, pyruvate, glycerol, malate, etc.). Also, the extent and nature of deficiency of oxidation of these intermedicate carbohydrates and of glucose and other mono- and di-saccharides are investigated by analysis of $^{14}CO_2$ and ^{3}HOH in the body water. These studies are done with human subjects, with rats in vivo, and with perfused rat liver. Other studies with labeled compounds are concerned with lipogenesis in liver and other sites. The effects of nutritional variations and of drugs affecting

14. Scope: (Cont'd)

endocrines and intermediary metabolism are further examined for diagnostic application and implications for therapy.

15. Relationship to Other Projects:

Related studies at BNL include those of Steele in the Biology Department and Dahl in the Medical Department. Dr. Steele's work on plasma glucose and lipid turnover in dogs using C-14-labeled glucose and lipids with effects of insulin and growth hormone has been related to some of the work with human subjects, but uses different technical approaches. Dr. Dahl has done some studies in hypertensive patients with glucose and fructose tolerance and concomitant insulin production (and hyperuricemia), which have indicated diabetic-like abnormalities. These studies have not used C-14- or H-3-labeled compounds.

Related studies are carried on elsewhere by E. S. Gordon, University of Wisconsin School of Medicine; C. Waterhouse, University of Rochester School of Medicine and Dentistry; G. Searle, V. A. Hospital, San Francisco; R. Kreisberg, University of Alabama Medical School; and M. Pollycove, San Francisco General Hospital. All of these investigators have been or are studying oxidation of glucose or other carbohydrates to $^{14}CO_2$ in human subjects in different ways; none is known to include glucose-C-14 in oral glucose loads or study labeled galactose, as done here with diabetic and obese patients.

Kreisberg, Searle, Waterhouse, G. Hetenyi (University of Toronto), and N. Kalant (University of Montreal), have measured glucose turnover and synthesis in humans (obese and/or diabetic) by similar techniques but none are doing combined studies in humans with carbon-14 and tritium-labeled carbohydrates.

J. W. Farquhar, Stanford University; E. L. Bierman, Seattle V. A. Hospital; E. A. Nikkila, University of Helsinki; S. Sailer, Universitats-klinik in Innsbruck, Austria; and I. MacDonald, Guy's Hospital Medical School, London, have done or are doing studies of lipogenesis with labeled carbohydrate precursors in patients with similar techniques. There are differences in choice of dietary regimen, labeled carbohydrates, classes of lipids examined, etc. None has planned (or reported) a specific study of interaction of dietary changes and oral contraceptives.

W. Malaisse, University of Brussels; F. Matchinsky, Washington University, St. Louis; G. Grodsky, University of California Medical Center, San Francisco; B. Hellman, University of Umea, Sweden, G. B. Boder, Lilly Research Laboratories, Indianapolis; and R. Luft, Karolinska Hospital, Stockholm; have examined insulin production in vitro from perfused pancreas or from isolated pancreatic islets. However, none has used a method of continuous flow of medium from incubated islets for serial analysis of insulin as developed here.

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16. Technical Progress in FY 1970:

As previously described, in collaboration with Dr. Brodoff, L-alanine-U-C-14 was given intraperitoneally to fasting Egyptian "sand rats" with varying degrees of carbohydrate intolerance which can be ameliorated by electrolytic lesions of the arcuate nucleus of the hypothalamus and by the anti-adrenergic drug, tetrabenizine. Conversion of alanine-C-14 to blood glucose was measured as an index of gluconeogenesis. The range of conversion was from 2 to 10% among all rats and there were no significant differences between non-treated rats and those with lesions of the arcuate nucleus or treated with tetrabenizine, which suggests that the mechanism of improvement of glucose tolerance is not by decrease of gluconeogenesis. Further studies directed toward elucidation of effects on glycogenesis and glycogenolysis were done with glucose-6-C-14 and are currently being assayed.

For the study of effects of hydrocortisone on gluconeogenesis from malate-C-14, H-3 in perfused rat liver, some final samples of blood glucose were analyzed to test in vivo vs. in vitro effects of hydrocortisone. Hydrocortisone has caused increased gluconeogenesis from malate both in vivo and in vitro while longer action in vivo promotes glycogenesis. A manuscript was prepared on studies carried out in Sweden on conversion of pyruvate-2-C-14 to breath $^{14}CO_2$ and blood glucose in acromegalic patients. Patients with decreased glucose tolerance displayed also a reduction in rate of oxidation of pyruvate-2-C-14 without change in gluconeogenesis from pyruvate.

In the study of conversion of glucose-1-C-14 to $^{14}CO_2$ during glucose tolerance test, the oxidation of the labeled glucose during standard vs. cortisone glucose tolerance tests were compared in seven obese or obese, diabetic patients. While the plasma glucose concentration (1 to 2 hours after oral load) was increased about 10% on the average by cortisone, the conversion of glucose-1-C-14 to $^{14}CO_2$ was increased by 20% at 1 hour and 10% at 2 hours. This suggests that the short-term hyperglycemic effect of cortisone in vivo in man is predominantly due to an increase of gluconeogenesis rather than a decrease of utilization of blood glucose. Glucose-1-H-3 was also given to some of these patients and glucose-2-H-3 to others. Usually the rate of conversion of H-3 to body water was also increased by cortisone, but the results were more variable than with C-14. Glucose-2-H-3 was converted to ^{3}HOH distinctly faster than glucose-1-H-3 and possibly by a two-phase process during the first 3 hours.

Since increase of plasma fucose has been reported to be excessive in latent diabetes, some plasma samples taken from patients during glucose tolerance test with glucose-1-C-14 were analyzed by gas chromatography of volatile carbohydrate derivatives to measure fucose and isolate it for radioactivity analysis. No increase of fucose during the glucose tolerance test was found.

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16. Technical Progress in FY 1970: (Cont'd.)

In the study of metabolism of glycerol-C-14, H-3 in obese, diabetic patients, comparison was made of the incorporation of tracer into plasma glycerides of these patients (6 females) vs. a group of 4 normal females. There are no significant differences between the groups for percent dose in total plasma triglycerides (TG) or di-+ mono-glycerides (DMG). For each group there is slightly more activity in the DMG than the TG. For the total glycerides the mean percent dose incorporation is less than 0.2%. Glycerol-C-14 and glycerol-H-3 show the same results, so there is no metabolic loss of H-3 before glyceride formation.

For the study of lipogenesis from different C-14-labeled saccharides (fructose, glucose, and sucrose) in normal rats, more accurate and detailed analyses have shown that the labeling of several types of lipids (glycerol and fatty acid moieties of triglycerides and di- + mono-glycerides, free fatty acids) of liver or plasma is 3 to 10 times higher from sucrose-C-14 than from glucose-C-14, with fructose-C-14 generally intermediate. This emphasizes a synergistic effect between glucose and fructose (the two moieties of sucrose) or else reflects more rapid absorption of the disaccharide (concomitant with hydrolysis by mucosal sucrase) than of either monosaccharide. Presently some lipid samples from rats given a mixture of labeled glucose and fructose (with an oral mixed load) are being analyzed to test the latter hypothesis.

Studies with C-14-labeled sucrose in patients have not yet been started, but non-isotopic sucrose tolerance tests in obese patients with analyses of the plasma curves of glucose and immunoreactive insulin (IRI) were done. Some patients seem to have higher insulin curves together with lower glucose curves than during glucose tolerance tests. This suggests special effects of sucrose and may help explain the high lipogenic potential of sucrose.

Studies initiated in patients using another labeled carbohydrate, galactose-1-C-14, which for theoretical reasons (particular inhibition of its oxidation by high reducing potential in the liver) could show differences in rate of formation of $^{14}CO_2$ between normal subjects vs. obese or diabetic patients greater than with labeled glucose. Study of one non-obese and three obese female patients so far are promising in this regard.

In the study of production and release of insulin by isolated rat pancreatic islets perfused by continuous flow of medium with serial analysis of IRI, the parameters of response to graded increase of glucose concentration and different initial and constant glucose concentrations were measured. Multiple major phases of release with fine peaks within the major cyclic release were revealed. Thresholds of response and saturating concentrations of glucose were defined and an initial paper has been submitted for publication.

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17. Expected Results in FY 1971:

Analyses of C-14 in glycogen from liver of Egyptian "sand rats" given glucose-6-C-14 will be completed to test whether the anti-adrenergic drug, tetrabenizine, improves glucose tolerance in this species by an effect on glycogen metabolism. If adequate personnel is available, the question of gluconeogenesis from L-alanine-U-C-14 might be reexamined with administration of the labeled compound during an oral glucose load.

The studies of oxidation of glucose-1-C-14 and glucose-1-H-3 to $^{14}CO_2$ and ^{3}HOH in obese, diabetic patients will be continued without the prior administration of cortisone before the glucose tolerance test. To the same patient (and a matching set of normal subjects) will be given galactose-1-C-14 in an oral galactose load to compare with oxidation of labeled glucose.

Studies of formation of liver and plasma lipids of normal rats from sucrose-U-C-14 compared with glucose-U-C-14 + fructose-U-C-14 will be further pursued to delineate reasons for particular lipogenesis from sucrose. The plasma insulin response of these rats to the different carbohydrate loads may also be studied.

A beginning will be made on study of lipogenesis from sucrose in patients given sucrose-U-C-14 in an oral load. This may be compared with glucose-U-C-14 (or glycerol-2-C-14) in the same patient. Further, if additional personnel are available, there may be a study of the effect of different oral contraceptives on the metabolism of labeled carbohydrates to plasma lipids and rate of turnover of the latter. This should help explain mechanisms of recently observed effects of estrogens in contraceptives to cause glucose intolerance, hyperlipemia, and hyperinsulinemia and could indicate possible synergistic effects of high sucrose diet and mixed contraceptives. Using fractionation of insulin on sephadex gel and/or disc-gel electrophoresis, it is hoped to study the output of insulin and its variants (e.g., proinsulin) into plasma of these patients during carbohydrate load and into the medium of perfused rat pancreatic islets.

18. Expected Results in FY 1972:

The studies will be consolidated and reported with studies in 06-03-01-(b)

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

See 06-03-01-(b)

20. Proposed Obligations for Related Construction Projects:

See 06-03-01-(b)