COLLEGE OF MEDICINE

MAILING ADDRESS:
RADIOISOTOPE LABORATORY
CINCINNATI GENERAL HOSPITAL
CINCINNATI, OHIO 45229

UCCM/RCC1.958005.010e

March 22, 1971 copy

Dr. Robert Loind
Defense Atomic Support Agency
Att: STND
Washington, D.C. 20305

Re: DASA 01-69-C-0131

Dear Dr. Loind:

Enclosed is a proposal for FY ~~72~~ 71 There have been only slight modifications in the research proposal but we plan to submit further plans and data prior to 1 May, 1971.

The budget request is in accordance with our previous requests.

We shall be happy to supply more information as you request.

Thank you for your interest.

Sincerely,

Eugene L. Saenger

ELS/rvl
encl:

Eugene L. Saenger, M.D.

Item 1. Subtask Number and Title: Radiation Effects in Man: Manifestations and Therapeutic Efforts NTER [redacted]009

Item 2. Agency Having Technical Supervision: Defense Atomic Support Agency, ATTN: [redacted]

Item 3. Agency Performing Work: Department of Radiology, University of Cincinnati College of Medicine: Contract No. DASA 01-69-C-0131

	Principal Investigator - Name
FY (PRIOR)	Eugene L. Saenger, M.D.
FY (CURRENT)	Eugene L. Saenger, M.D.
FY (+1 Budget)	Eugene L. Saenger, M.D.

Item 4. Funding Data:

FY (Prior)	$ 71,369
FY (Current)	80,000
FY (+1 Budget)	93,232
FY (+2 Planning)	96,000
FY + 3	98,000
FY + 4	100,000

Item 5. Estimated Completion date: 1975

Item 6. Requirement and/or Justification:

As nuclear weapons are deployed about the world in increasing numbers and as the peaceful use of atomic energy similarly spreads, the need to acquire detailed knowledge about human radiobiology becomes more and more urgent. It is in this direction that our research efforts have been concentrated for a number of years. It has become increasingly apparent that extrapolation of radiation results from animals to man is of only limited value and therefore we have concentrated upon radiation effects in patients, with only limited animal experimentation.

The proposed investigations contained herein continue our acquisition of [redacted] on clinical manifestations of radiation injury, alterations in [redacted] metabolism, evaluation of bone marrow reserves after irradiation, and [redacted] logical effects of radiation especially on cognitive functions. Studies newly undertaken include evaluation of certain serum enzymes and of chromosome aberrations as biological radiation dosimeters, the use of tritiated thymidine incorporation into lymphocytes for the same purpose, and electron microscopic investigations of radiation damage in human leukocytes

Incl 1

69-C-0131-P00003

Cooperative studies with AFRRI will continue for investigation of plasma bound neutral hexoses as predictors of radiation sensitivity. Our own studies of glycoprotein changes after irradiation will also be pursued. We shall also continue to examine the effects on our biological radiation dosimeters of burns and trauma to ascertain that our findings are specific for radiation injury, since soldiers in nuclear warfare may be exposed to all three types of injury.

These studies are performed, as noted in previous years, on patients who are given total and partial body irradiation as treatment for metastatic malignancy in place of systemic chemotherapy where the latter is of questionable value. The study design has been approved by the Research Committee of the College of Medicine in regard to its therapeutic value and informed consent of all subjects so treated has been obtained.

Several areas of investigation will continue. These include an approach to the prevention of the graft versus host syndrome in homologous bone marrow transplantation now that the autologous marrow transplant has proven successful in our hands. We will continue to investigate the ability of a synthetic chemical diet to alter the acute radiation syndrome in man, correlating this with the alterations which this diet causes in fecal flora. In general the pattern of studies will continue as in the previous year with particular attention being given to trunk irradiation and upper body irradiation as well as exposure to the whole body in order to obtain better information concerning human radiation effects.

Item 7. Research Work Units:

A. Identification

1. a. DASA 01-69-c-0131. Radiation Effects in Man: Manifestations and Therapeutic Efforts

2. Department of Radiology

3. Principal Investigator: Eugene L. Saenger, M.D.

Collaborators: Bernard Aron, M.D.
I-Wen Chen, Ph.D.
Goldine C. Gleser, Ph.D.
Harry Horwitz, M.D.
James Kereiakes, Ph.D.
Edward B. Silberstein, M.D.
Carolyn Winget, M.A.

Consultants: Louis Gottschalk, M.D.
Evelyn Hess, M.D.
Robert Kunkel, M.D.

4. Continuation of Existing Work Unit: Yes

5. Estimated Interim Report Date: 1971

6. Estimated Completion Date: 1975

7. Estimated Final Report Date: 1975

8. If R & D Contract Proposal:

Cost $93,232
Start Date: 1971
Completion Date: 1972
Agency: University of Cincinnati College of Medicine, Cincinnati General Hospital, Cincinnati, Ohio 45229
Continuation of Existing Contract: Yes

Synopsis of Research Proposal:

These studies are designed to obtain data from whole and partial body radiation given for therapeutic purposes in the human being. The effects being studied are clinical, hematological, biochemical, and behavioral.

9. Approach:

a. Selection of Patients

Patients selected for study will be those who are to receive partial or total body irradiation in the treatment of metastatic malignancy from various primary sites. These primary sites exclude neoplasms which may be treated by internal radiation such as carcinoma of the thyroid or prostate and tumors for which there is effective systemic chemotherapy, such as carcinoma of the breast and prostate. These patients will have stable hematologic values although they may have received local radiation previously.

b. Experimental Design

Each patient serves as his own control in our study. This is possible because the patients are stable both clinically and in respect to all laboratory data. Two to four observations are made in the pre-irradiation period and then the patient receives one or two days of sham irradiation. Post-irradiation observations are then made at intervals of one to three days until clinical and laboratory values become stable.

Some of the patients will be hospitalized on the Tumor or Medical Wards of the Cincinnati General Hospital and others will be placed in the Clinical Research Center of this newly constructed facility. In many situations the irradiated individual will be followed entirely on an out-patient basis. Since whole-body radiation is occasionally utilized in children, an opportunity to study such patients in the Clinical Research Center of Children's Hospital occurs about 1-2 times per year.

During the pre-irradiation period, the patient's records are reviewed by the physicians to be certain that the contribution of the underlying disease can be evaluated accurately. One or two sham irradiations are given to permit accurate dosimetry, obtain cooperation by the patient and evaluate psychological factors induced by the environment of the irradiation facility.

The sham treatments will be followed immediately by the whole or partial body irradiation procedure employing teletherapy. There is no discussion of possible subjective reactions resulting from the treatment. Other physicians, nurses, technicians and ward personnel are instructed not to discuss symptoms or reactions with the patient.

A new 4 MeV linear accelerator will be installed. Special provisions have been made to carry out whole and partial body radiation and higher dose rates will be possible.

c. Parameters to be Investigated

1. Clinical Findings

(a) Complete history and physical examination
(b) Temperature, pulse and respiration
(c) Body weight at standard test times

2. Hematology and other Laboratory Tests

(a) Hgb., RBC, WBC, differential, hematocrit, platelets, reticulocytes, erythrocyte sedimentation rate, leukocyte reaction to etiocholanolone to evaluate bone marrow reserves.

(b) Bone marrow--smear and block for microscopic pathological analysis at regular intervals.

(c) Urinalysis

(d) BUN, FBS, sodium, potassium, chloride, CO_2 alkaline phosphatase (total), LDH (total), SGOT, calcium, phosphorus, albumin, serum globulin.

(e) Selected enzymes and other less frequently measured serum constituents including gastrointestinal alkaline phosphatase, LDH isozymes, amylase isozymes, muramidase, creatine phosphokinase, serum histamine, serum transferrin, serum 5-hydroxyindole acetic acid, and pre- and post-radiation karyotyping for biologic dosimetry.

(f) Tritiated thymidine uptake by lymphocyte suspensions pre- and post-radiation.

(g) Histochemical studies.

(h) Electron microscopic studies of leukocyte damage.

3. Biochemical studies including tracer studies of isotopically tagged nucleosides in treated humans. Comparison with patients suffering burns and other trauma.

4. Bone marrow autotransplantation with animal study of homotransplantation and prevention of graft versus host disease.

5. Use of a chemically defined synthetic diet to alter the gastrointestinal radiation syndrome and measure changes in fecal bacteria.

6. Psychiatric evaluation and testing.

   (a) Five minute verbal samples
   (b) Clinical psychiatric interviews and rating scales

Dosimetry

In previous studies the radiation beam has been directed horizontally at a wall 338 cm away with the patient midline at 282 cm from the source. Under these circumstances the beam area for the 50% isodose curve at the patient's midline has been a square field 75 x 75 cm. The patient is placed in a sitting position with legs raised and head tilted slightly forward. Each lateral portal receives half the dose.

A new Cobalt 60 teletherapy unit (Theratron Model 8, Atomic Energy of Canada, Ltd) was installed. The dose rates as used in the past of 3-6 R/min will continue. The source to midline-trunk distance for this unit is 286 cm. The field size at 286 cm for this unit is 100 cm (90% isodose line) compared to 75 cm (50% isodose line) for previous Eldorado 8 Unit, thus providing a larger and a more homogeneous radiation field.

Dosimetry studies are in progress to determine experimentally "active" bone marrow dose under simulated whole-body and half-body Co-60 exposure conditions. Thermoluminescence dosimeters (LiF) are being placed in selected bone spaces of an Alderson Rando phantom and the phantom then irradiated under simulated patient exposure conditions. These "active" bone marrow doses will be related to the hematologic findings observed for whole-body irradiation and for other conditions of partial body irradiation. These data will aid in interpretations of the effects of shielding portions of "active" bone marrow.

Clinical and laboratory observations will continue as indicated above. There will be 3-5 observations made before radiation, daily

observations afterwards for 3 days and then every 3 days as long as deemed necessary.

Comparisons will be made for partial vs. whole body, partial upper vs. partial lower, and trunk (sternum to pubis) vs. partial irradiation of upper and lower body. The frequency of prodromal symptoms, hematological changes and biochemical changes will be followed.

As indicated above, the doses for partial body radiation will be increased in comparison to those used previously since the earlier dose levels have been well tolerated. Comparison between patients will indicate trends in relation of different dose levels to prodromal symptoms. In previous studies it has been noted that clinical symptoms with partial body radiation are proportional to the dose received although hematological changes are either minimal or absent. In one patient receiving 300 rads of partial body radiation, a significant drop in white cell elements were noted. These changes will be evaluated in reference to bone marrow reserves by etiocholanolone. Since some of these patients experience therapeutic benefit from the radiation, the use of higher doses of partial body irradiation is both justified and desirable. This effort will seek to identify the dose range causing hematological changes similar to those observed with previously observed levels of whole body radiation.

Hematology

1. Etiocholanolone

Since February 1969 we have been employing etiocholanolone, a naturally occurring steroid metabolite, to measure the bone marrow reserves of patients receiving whole-body irradiation. This hormone injected intramuscularly causes a leukocytosis as granulocytes enter the peripheral blood stream from the bone marrow. The normal increment in the total granulocyte count (neutrophils, stab cells, eosinophils, basophils, plus some less mature forms) exceeds 2,600 cells per cu/mm within 16 to 24 hours after a dose of etiocholanolone 0.10 mg. per kg body weight is injected.

Although radiation-induced leukopenia has been observed for many years, the decrease in granulocyte count is not immediate. However, our preliminary data indicate that bone marrow reserves of granulocytes are decreased while the patient's WBC is still within normal limits. We have also been able to observe a cyclic variation in the granulocyte reserve response after irradiation, with which we feel we will be able to explain the partial marrow recovery noted frequently within two weeks after radiation injury. Further study in this area will permit greater definition of the value of etiocholanolone in evaluation of marrow damage caused by partial as well as whole body irradiation.

2. Chromosome Studies

There continues to be some controversy as to the precise power function by which radiation dose is related to aberration yield. With the accurate dosimetry available on our patients at the Cincinnati General Hospital we plan to continue our study of chromosomes as a biologic dosimeter and redefine our dose-response curve with greater precision. Much older data has depended on 72 hour lymphocyte cultures whereas our more recent technique, involving 48 hour lymphocyte cultures after phytohemagglutinin stimulation should provide clues to some unanswered questions.

The specificity of chromosome aberrations, ring and dicentric formation, as related to radiation damage has never been questioned. It is known that severe burns may alter the membranes of the circulating cells and that red blood cells may also be injured by trauma, as in "march hemoglobinuria". Perhaps this is true of lymphocytes as well. We plan to continue karyotyping selected patients referred to our large Shriner's Burn Hospital to assess the frequency of chromosome breakage in these patients. We plan similar studies in patients suffering severe trauma in cooperation with the Department of Surgery when we have adequate personnel for this purpose.

3. Tritiated Thymidine Studies

We have initiated the study of incorporation of tritiated thymidine into lymphocytes before and immediately after whole-body irradiation. We plan to extend these studies to note the time course during which the incorporation of tritiated thymidine becomes normal and to evaluate the value of this test as a biologic dosimeter. Since the lymphocyte is such an exquisitely radiosensitive cell, thymidine incorporation may be a dosimeter of injury over a wide spectrum of radiation dose.

4. Electron Microscopy and Histochemistry

Because of the wide range of histochemical tests available, one may study the appearance of the different subcellular organelles under the light microscope. These include mitochondria, ribosomes, lysosomes, as well as the cellular content of glycogen, various fats and the phagocytic ability of cells. A more basic approach however, is first to analyze the pathologic correlates of radiation injury with the more sensitive electron microscope. After we have identified which, if any, of the organelles are affected by the radiation doses employed we may then apply the appropriate histochemical stains to determine if this damage seen on the electron microscope can also be picked up by tests for the integrity of that cellular component which would be available in the field. Among the histochemical tests available include stains for glycogen, alkaline phosphatase, acid phosphatase, beta glucuronidase, periodic acid-Schiff reaction, PAS-diastase, methyl-green-pyronin, Sudan black, peroxidase, lactic acid dehydrogenase, succinic acid dehydrogenase, dihydro-orotic acid dehydrogenase and several others.

5. Marrow Transplantation

Recent work in this laboratory has proven the efficacy of our technique of human marrow transplantation (both iso-transplantation and auto-transplantation) with greater prevention of radiation-induced leukopenia than has heretofore been reported. We plan to pursue these studies of marrow transfusion as protection against radiation injury at higher doses of radiation now that our technique has been proven effective at 200 rads. A single patient who received marrow auto-transplant after 250 rads whole-body irradiation has also been protected from severe radiation leukopenia through this technique. The reduction in the incidence of lymphocyte chromosome aberrations after marrow transplantation will also permit theoretical calculations of lymphocyte kinetics in our irradiated patients.

Biochemical Studies

1) Possibility of enzyme analysis to determine distribution of radiation dose to human body.

It seems possible to use enzymes arising in various organs to indicate which portions of the body are irradiated. Thus estimates of dose and distribution may become available by "enzymatic biopsy". The approach to this type of evaluation is essentially the same for each enzyme. The enzyme response will be followed in subjects where the relevant tissue or organ is irradiated. Their response will be compared to that of subjects whose radiation is given to tissues wherein the enzyme of interest is not found. Dose response curves will also be sought.

In our recent studies with amylase activities in serum and urine, we found that 6 out of 10 cancer patients showed significant increase in their serum amylase levels (5 to 20-fold increase) after total-body irradiation of 100-200 rads. The other four patients also showed an increase in this enzyme activity but the increase was not significant statistically because of the wide range of amylase level in normal serum.

The increase in the serum amylase level of cancer patients irradiated above the head and neck have been reported by several investigators (1,2). They have shown that the increase in the amylase activity is due to the release of salivary amylase in the blood and that the amylase present in the normal serum represents the amylase from the pancreas. The pancreatic and salivary amylases can be separated by electrophoresis. Recently, a technique involving electrophoresis in polyacrylamide gel slabs has been developed to isolate many amylase isozymes (3). This technique will be used to determine salivary amylase activity in the serum and urine of cancer patients before and after irradiation. Patients selected for this study will be those who are to receive partial or total body irradiation in the treatment of metastatic malignancy from various primary sites or out-patients of our therapeutic division receiving local irradiation for the treatment of head and

neck tumors. Dose-response study will also be carried out on selected patients in our therapeutic division who will receive initial treatment doses of 50, 100, 150, 200, 250 and 300 rads for head and neck tumors. The detection of salivary amylase in serum and urine could prove to be a sensitive biological indicator of radiation dose since salivary amylase is not present in the normal serum or urine and could be used to determine the radiation dose on the head and neck.

Recently, Muggia et al. reported a rise in serum CPK of patients receiving irradiation to major portions of the heart (4). The increase in this enzyme after irradiation could be used to determine the radiation dose on the chest. Both the patients with total body irradiation and those with partial irradiation including the chest will be used in this study.

Alkaline phosphatase isozymes have been reported to increase in serum of patients with liver and bone disorders (5). Intestinal mucosa is rich in this enzyme and is known to be extremely sensitive to ionizing radiation. Therefore, the changes in these isozymes might be used to determine the distribution of radiation dose on the abdominal portion of a human body.

The change in the pattern of lactic acid dehydrogenase isozymes has also been reported in monkeys after receiving γ-irradiation and this will also be investigated (6).

2) Radiation effects on the metabolism of nucleosides

In our previous studies with isotopically labelled CdR, it was shown that radiation could alter the metabolism of CdR in rats (7). It is important to find out whether irradiation will alter the catabolic patterns of CdR in humans as well, because the results of this study may lead us to the discovery of urinary compounds as yet unidentified which can be used as sensitive biological indicators for radiation dose in man.

The methodology is similar as described in the previous proposal. Five to ten microcuries of sterilized and pyrogen free ^{3}H-5-CdR will be given intravenously before and after the radiation therapy. The time between first and second injection will be at least 5 days. The excreted radioactive compounds will be analyzed by column and paper chromatography. The quantity of CdR injected is negligible (less than 0.2 ug per injection) and dose of 5-10 microcurie of radioactivity are below MPD levels for the population at large.

In addition to CdR, radiation effects on metabolism of other isotopically labelled nucleosides such as thymine, uridine and deoxyuridine will be studied. Studies of uridine metabolism are especially interesting because uridine is known to be converted to pseudo uridine by man (8). Pseudo uridine has been detected in human urine (9) and urinary excretion of this compound is shown

to increase in man after irradiation (10).

References

1. Van der Brenk, H.A.S., Hurley, R.A., Gomez, C., and Richter, W. Serum amylase as a measure of salivary gland radiation damage hyperamylasaemia following fractionated exposure to 4 MeV X-rays delivered in high pressure oxygen, and effects of certain steroids on this response. Br. Jour. Radiol. 42:688 (1969).

2. Kashima, H.K., Kirkham, W.R., and Andrews, R.J. Post-Irradiation Sialadenitis: A study of the clinical features, histopathologic changes, and serum enzyme variations following irradiation of human salivary glands. Am. J. Roentgenol. 94:271, (1965).

3. Boettcher, B., and de la Lande, F.A., Electrophoresis of human salivary amylase in gel slabs. Analytical Biochemistry 28:510, (1969).

4. Muggia, F.M., Ghossein, N.A., and Hanok, A., Creatine phosphokinase and other serum enzyme during radiotherapy. J. Am. Med. Assoc. 211: 1345, (1970).

5. Kaplan, M.M., and Rogers, L., Separation of human serum - Alkaline phosphatase isozymes by polyacrylamide gel electrophoresis, Lancet No. 15, 1029, (1969).

6. Hawrylewicz, E.J., and Blair, W.H., Enzyme isoenzyme measure of radiation exposure. Aerospace Medicine 38:30, (1967).

7. Chen, I.W., Wrede, D.E., Kereiakes, J.G. and Saenger, E.L., Radiation effect on the metabolism of isotopically-labelled deoxycytidine in rats. Radiation Research 39:490, (1969).

8. Weissman, S.M., Lewis, M. and Yaron, M., The metabolism of isotopically labelled uracil and uridine in man. Metabolism 12:60, (1963).

9. Adler, M. and Gutman, A.B. Uridine isomer (5-ribosyl-uracil) in human urine. Science 130:862, (1959).

10. Drahovsky, D., Winkler, A. and Skoda, J. Urinary excretion of pseudo-uridine in the course of X-ray therapy. Neoplasma 12:561, (1965).

Psychological Evaluation

Following exposure to acute whole- or partial-body radiation, it is possible that there will be significant impairment of the decision-making capability of key personnel who have major command responsibilities. This concern has become more important in recent years since the findings that complex electronic systems can be rendered inoperative by very high doses of radiation. Thus it is necessary to maintain dependence on the human being. It is quite possible that even moderately high doses or dose rates could produce impairment of cognitive processes either of an obvious or of a subtle nature which in moments of stress would impair or defeat a military operation. In order to gain understanding of such possible changes it is necessary to seek changes in cognitive processes and decreases in the capability to perform highly technical processes.

In conjunction with the Department of Psychiatry a continuing program for assessing the effects of total and partial body radiation on cognitive-intellectual functioning and emotional reactions has been carried out. The procedure includes an initial series of interviews and tests in order to evaluate the mental status of the patient and to gain some understanding of the patient's reaction to his condition and to the intended treatment. A Wechsler-Bellevue intelligence test and the Cattell 16 Personality Factor Questionnaire are given during this initial assessment period. In addition, the patient is introduced to the procedures which will be utilized on subsequent testing occasions: recording a five-minute verbal sample in response to standardized instructions, the Reitan Trail-Making Test, and the non-structured interview on the basis of which a Wechsler Depression Scale is filled out. The patient is then assessed on twelve additional occasions: pre- and post-sham radiation; pre- and post-radiation, 1 day, 3 day, and weekly thereafter for six weeks.

The typescripts of the verbal samples compose the raw data from which scores on transient cognitive impairment, anxiety, hostility, and hope are obtained using the methods of content analysis developed and validated by Gottschalk and Gleser in the Department of Psychiatry at the University of Cincinnati.

As an increasing amount of data are accumulated it is possible to intensify the focus on variables differentiating those receiving high total-body radiation from those receiving either low total-body radiation or partial-body radiation. Certain trends have begun to emerge. Further data analysis will be aimed at using these trends and possibly obtaining others to develop a multiple regression equation to predict the amount and type of radiation

BUDGET

Personnel	% total effort	Amount
Internist	33%	$ 5,500
Radiation therapist	5%	1,500
Physicist	4%	1,000
Biochemist	90%	15,000
Technician	100%	7,000
Technician	100%	7,000
Laboratory aide	50%	2,000
Medical Illustrator		650
Secretary	66%	5,000
Psychiatrist	8%	1,500
Research Psychologist	37%	4,100
Research Technician	50%	3,500
	Salaries	53,750
	13% staff benefits	6,988
Consultant		1,000
Equipment		1,000
Consumable supplies		5,500
Travel		2,000
	Total direct costs	70,238
	(Overhead (42.78 salaries)	22,994
	TOTAL	$ 93,232