Item 1. Subtask Number and Title: Radiation Effects in Man: Manifestations and Therapeutic Efforts NMLR R&D 3.009

Item 2. Agency Having Technical Supervision: Defense Atomic Support Agency, Attn: STND

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

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	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 (total funding up to Prior FY)	$ 335,556
FY (Prior)	71,326
FY (Current)	71,369
FY (+1 Budget)	93,232
FY (+2 Planning)	94,000
FY + 3	96,000
FY + 4	98,000

Item 5. Estimated completion date: 1975

Item 6. Requirement and/or Justification:

As noted regularly world tensions seem to increase each year as does the possibility of nuclear warfare perhaps instigated by a "new" nuclear power.

With the additional serious military and domestic problems of the United States it seems desirable to intensify efforts directed toward nuclear weapons effects in directions will will yield information of practical value and of possible direct application to human beings. It is to these goals that the following proposals are being structured. As before the major effort is directed toward human studies. Occasional animal studies are utilized for preliminary investigation as needed.

The proposed studies continue investigations of clinical manifestations, changes in metabolism of DNA and RNA, evaluation of bone marrow reserve, delayed hypersensitivity, various aspects of marrow transplants, psychological effects and cognitive functions. We propose some new evaluations of chromosome changes, use of thymidine incorporation into lymphocytes, histochemical investigations of leukocyte physiology and evaluation of certain new serum enzymes.

69-C-0131-P001 Progress

Cooperative studies with AFRRI will continue for investigation of plasma bound neutral hexoses as predictors of radiation sensitivity.

Because of interest in this laboratory in combined effects, certain preliminary studies are being initiated to determine possible similarities in body responses of deoxycytidine excretion and chromosome changes in comparing irradiated and burned patients. Certain patients undergoing severe trauma will also be studied. The possible similarities in physiological response to different physical agents has not been investigated previously. This approach may increase our understanding of possible interactions of different types of injury.

These studies are performed on patients who are given total or partial body radiation as treatment for metastatic malignancy. This therapy is being used in place of systemic chemotherapy. In the patients where radiation of this type is used our data suggests that it may be equal or superior to current chemotherapeutic measures. 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 the subjects under study.

The use of marrow transfusion, initially autologous, will be used both for support of the irradiated subject and as a possible important future method of treatment. With the rapid increase of understanding of tissue compatibility and techniques for analysis of these factors, the possibility of the use of homologous marrow even only as a temporary support until regeneration of an individual's own tissue becomes of increased interest.

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: Harry Horwitz, M.D.
James Kereiakes, Ph.D.
Edward B. Silberstein, M.D.

Consultants: Louis Gottschalk, M.D.
Evelyn Hess, M.D.
Robert Kunkel, M.D.
John Warady, Ph.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, immunological and behavioral. Evaluation of storage and reinfusion of autologous bone marrow is being carried out.

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, excluding neoplasms which are treated by internal radiation, e.g. carcinoma of the thyroid. They will have stable hematologic values and may have received local radiation previously.

b. Experimental Design

The design of the study is such that the patient serves as his own control. For this contract period there will be two groups. In the first, pre-therapy studies will be completed within one week and immunological studies will be omitted. It has been found that too many subjects were lost either for personal or medical reasons because of the 21 day pre-irradiation study period. A second group for patients who can tolerate the 21 day pre-irradiation will be continued whenever possible. The post-irradiation period studies continue as long as the clinical course of the patient allows. Because of the careful design of the study it is possible to make between patient comparisons.

Three to five observations are made in the pre-irradiation period. Post-irradiation specimens are then obtained at three day intervals or oftener until clinical and laboratory findings become stable.

Some of the patients will be hospitalized on the Tumor and Surgical Wards of the hospital. Other patients will be in the enlarged Clinical Research Center within the new Cincinnati General Hospital. The new hospital building will begin receiving patients in mid June 1969. In some situations the irradiated individual will be followed on an out-patient basis.

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. Two or more sham irradiations are given to permit accurate dosimetry, obtain cooperation by the patient and evaluate psychic factors. The sham treatments will be randomized between the actual radiation. 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.

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, Etiocholanolone for marrow reserve
   (b) Bone marrow--smear and block for microscopic pathological analysis at regular intervals
   (c) Urinalysis
   (d) Other clinical laboratory tests, e.g. Serum urea Nitrogen, electrolytes, etc., will be obtained if needed
   (e) Pre- and post-radiation karyotyping for biologic dosimetry.
   (f) Tritiated thymidine uptake by lymphocyte suspensions
   (g) Histochemical studies

3. Biochemical studies including tracer studies of isotopically tagged nucleosides. Additional enzyme studies. Comparison with patients suffering burns and other trauma.

4. Bone marrow storage and reinfusion

5. Immunology

6. Psychiatric evaluation and testing

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

Work Units:

This program is based on the concept of obtaining all possible useful information from each subject studied during the course of his therapy. Although this statement may seem to be a truism, one should understand that appropriate subjects are somewhat difficult to obtain as compared to non-human studies and that once a given subject is started it is desirable to derive all possible data for the effort required.

There are two major closely related work units:

A) Radiation exposure, clinical and laboratory evaluation and biochemical and immunological studies

B) Psychiatric and psychological studies

Work Unit A:

The techniques of administration of radiation will be as follows:

1. Bidirectional radiation. Half of the dose is given from each side as before.

   a. Whole body
   b. Partial body using the xiphoid as the boundary
   c. Trunk and thorax radiation using the suprasternal notch and symphisis pubis as the boundary

2. Unidirectional radiation. In selected instances the total dose will be given from an anterior, posterior or single lateral field.

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 vs. partial irradiation. 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 (10) 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 rad 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, eosinophils, basophils, plus some less mature forms) exceeds 2,600 cells per cu/mm within 16 to 24 hours after

0.10 mg. of etiocholanolone per kg/ body weight is injected intramuscularly.

Although radiation-induced leukopenia has been observed for many years, the decrease in granulocyte count occurs days to weeks after radiation exposure. Immediate alterations in granulocyte kinetics after irradiation have not been previously studied. Preliminary data suggest that the granulocyte reserves are significantly depleted within 24 hours of a wholebody dose of 200 rads but not by 100 rads. At this time the peripheral blood counts remain within normal limits. Further study in this area will provide important information concerning the use of granulocyte reserves as a biologic dosimeter as well as yielding valuable data concerning the pattern of post-irradiation marrow recovery.

2. Chromosome Studies

Difficulties with dosimetry in human beings irradiated under conditions of war or accident have hampered efforts to employ radiation-induced chromosome aberrations as a biologic dosimeter. There is thus some controversy as to the precise arithmetic function by which radiation dose can be related to chromosome abnormalities. With the accurate dosimetry available on our patients at the Cincinnati General Hospital we plan to reopen our study of chromosomes as biologic dosimeters and to redefine the dose-response curve of chromosome breaks with greater precision. This material has been obtained and available on about 60 patients and includes at least 3 pre-irradiation and at least 10 post-irradiation observation days.

Combined burn, trauma and radiation injury is likely to occur in any nuclear holocaust. However the specificity of chromosome abnormalities as related to radiation damage has never been seriously questioned. It is well known that severe burns may effect red cell stroma, and perhaps lymphocytes may also suffer heat-induced damage. Similarly, red blood cells may be damaged by severe trauma, as in "march hemoglobinuria", and by anology perhaps lymphocytes as well. Thus we plan to perform karyotypes on 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.

3. Tritiated Thymidine Studies

Tritiated thymidine incorporation by animal small intestine obtained after radiation is depressed. This sort of study is clearly impractical in the field. We plan to study tritiated thymidine incorporation by lymphocyte suspensions of our patients before and immediately after whole-body irradiation. 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. Histochemistry

Several leukocyte enzymes and other biochemical constituents may be studied with appropriate histochemical techniques to evaluate radiation-induced functional and structural alterations. These stains may be applied to fresh blood smears in the field to obtain data rapidly related to radiation dose. Preliminary studies in this approach to cell physiology are now underway. We hope to study a number of cell constituents, including glycogen, alkaline phosphatase, acid phosphatase, beta glucuronidase, periodic acid-Schiff reaction, PAS-diastase, methyl-green-pyronin, sudan black, peroxidase, lactic acid dehydrogenase, succinic acid hydrogenase, dihydro-orotic acid dehydrogenase as well as phagocytic ability. Preliminary studies in this approach to cell physiology are just beginning.

5. Marrow Transplantation

Marrow homotransplantation remains a hazardous procedure because the frequent resultant graft versus host disease is often lethal. A recent apparent successful marrow iso-transplant has given this laboratory an opportunity to refine considerably the appropriate techniques. Animal studies are planned to inactivate selectively immunologically competent marrow cells without altering the donor marrow stem cell function and thus pave the way for safe marrow homo-transplantation.

Our tumor patients are often not candidates for marrow auto-transplantation after whole-body irradiation because there are frequently marrow metastases present. By screening potential marrow sites with strontium-85 bone scans as well as by several marrow aspirations from these sites we hope to perfect the technique of auto-transplantation without reimplanting our patient's cancers.

Biochemical Studies

I. Radiation effects on the metabolism of isotopically labelled nucleosides.

1. Animal studies

Our previous studies have shown that the amount of deoxycytidine (CdR) excreted in urine of X-irradiated rats is proportional to the amount of radiation exposure up to 200 R but CdR-uria in man is not sensitive to radiation (1). We have also demonstrated that CdR is actively deaminated in human serums but not in rat serums (2). These results suggest that CdR is metabolized differently in these species and prompted us to study the effect of radiation on the metabolism of CdR specifically labelled with H-3 on the carbon-5 of the cytosine.

It was found that irradiated rats (200R) excreted about 21% of total radioactivity injected whereas only 13% of radioactivity

was excreted by unirradiated rats. Specific radioactivity of CdR isolated from the urine of irradiated rats decreased about 2 to 6 folds as compared with that of CdR in the urine of unirradiated rats, indicating the increase in the pool size of free CdR in irradiated rats. This result strongly suggests that the radiation-induced increase in urinary CdR is not due to a renal factor involving the defective tubular reabsorption of CdR. The radioactive compounds excreted in urines were analyzed by the use of ion exchange columns and paper-chromatography. Our preliminary results indicate that the urine of irradiated rats contained a radioactive compound or compounds which chromatographically behaved differently from the radioactive compounds isolated from the urine of unirradiated rats.

In the course of this study, we found that the amount of radioactivity excreted by rats varies considerably. In order to obtain a more precise comparison between unirradiated and irradiated rats, it is desirable that the same rats can be used as a control as well as an experimental animal. This is especially true for the experiment with humans because it can reduce the number of people receiving radioactive materials. The result of this study indicate that the same animal can serve as its own control. The radioactivity excreted into urines decreased to about 1% 24 hours after the injection of radioactive CdR. Five days later, the rats were injected with a second dose of radioactive CdR and irradiated. The post-irradiation urinary radioactivity was approximately doubled as compared with the pre-irradiation value. It was found in control experiments that the first injection of radioactive CdR did not affect the excretion pattern of urinary radioactivity from the second injection and that irradiation 5 days after the first injection did not alter the amount of urinary radioactivity from the first injection (3).

Identification of the radioactive compounds excreted in the urine is underway at present. Excretion of labeled compounds in urines of rats received radioactive CdR at various times in respect of the time of irradiation will also be studied.

2. Studies with humans

It is of extreme importance to find out whether irradiation will alter the catabolic patterns of CdR as found in the experiments with rats; because the results of this study may lead us to the discovery of urinary compounds which can be used as sensitive biological indicators for radiation dose in man.

Cancer patients with normal hepatic and renal function will be selected in this study. Five to 10 microcuries of sterilized and pyrogen free ^{3}H-5-CdR will be given intravenously before and after the radiation therapy. The time of 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 doses of 5-10 microcurie of radioactivity are below MPD levels for the population at large.

In addition to CdR, radiation effects on the metabolism of other isotopically labelled nucleosides such as thymidine, uridine, and deoxyuridine will be studied. Studies of uridine metabolism is especially interesting because uridine is known to be converted to pseudouridine by man (4). Pseudouridine has been found in human urine (5) and urinary excretion of this compound is shown to increase in man after irradiation (6).

II. Various enzyme activities in serum

It has been shown that many enzyme activities, especially the activities of lysosomal hydrolases such as DNase I, DNase II, phosphatases, beta-glucuronidase, and beta-galactosidase, etc, are elevated by x-ray irradiation. Our previous work has shown an increase of CdR deaminase activity in three cancer patients after irradiation (3). Other enzymes such as those mentioned above will be analyzed before and after therapy to determine the usefulness of these enzymes to serve as biological indicators of radiation damage.

III. Plasma protein bound neutral hexose

Recent studies of plasma concentration levels of protein-bound carbohydrates (PBC) in irradiated mice and dogs revealed that the animals which died of radiation exposure exhibited a marked increase in PBC while the survivors deviated only slightly from their pre-irradiation values (7). It was suggested that plasma concentration of PBC might provide a crude index of radiosensitivity prior to and prognosis after exposure to ionizing radiations in otherwise healthy individuals (8). It is of great importance to see whether the similar results can be found in humans because PBC level in plasma might be useful in screening individuals to be engaged in work involving a high risk of radiation exposure.

PBC levels in plasma of cancer patients before and after radiation therapy will be assayed in collaboration with Adelbert S. Evans of the Armed Forces Radiobiology Research Institute, Defense Atomic Support Agency, Bethesda, Maryland.

B. Psychiatric 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 decrease in the capability to perform highly technical processes.

These problems are difficult to solve. The studies can only be carried out using human subjects. There are relatively few tests which seem capable of yielding useful information.

In cooperation with the Department of Psychiatry a program has been started which may yield some of these answers. The tests are administered in the same way as in Work Unit A, i.e. in the pre- and post-radiation periods. During the pre-radiation period the tests are done in conjunction with the episodes of sham irradiation.

Certain psychological tests are performed in order to obtain an estimate of the subject's intelligence and personality structure. These include the Reitan Trail Making Test, Wechsler-Bellevue Intelligence Scale, the PAT and Par test.

Changes in cognitive thought processes are being studied by a method of verbal sampling. This technique is particularly useful since it requires no training or adaptation by the subject. At each interview the subject merely talks for five minutes about anything of interest or concern and the material is recorded on tape for subsequent analysis. These interviews are then analyzed for depression, hope, anxiety and clinical impression.

Two such interviews are obtained prior to any irradiation event. They are obtained immediately before and after each sham irradiation and similarly at the time of actual exposure. The tests are administered again two weeks after radiation.

These studies will then be analyzed to determine changes with dose and with total vs. partial body radiation and for differences between upper and lower body radiation.

Bibliography

1. Chen, I.W., Kereiakes, J.G., Friedman, B.I. and Saenger, E.L. Radiation-induced urinary excretion of deoxycytidine by rats and humans. Radiology 91:343, 1968.

2. Chen, I.W., Minton, S., Kereiakes, J.G. and Saenger, E.L. Deoxycytidine deaminase activity in serum of irradiated cancer patients. Presented at the 54th Annual Meeting of the Radiological Society of North America, December 1968.

3. Chen, I.W., Mrede, D.E., Kereiakes, J.G. and Saenger, E.L. Radiation effect on the metabolism of isotopically-labelled deoxycytidine in rats (abstract). Radiation Research, in press.

4. Weissman, S.M., Lewis, M. and Karon, M. The metabolism of isotopically labeled uracil and uridine in man. Metabolism 12:60, 1963.

5. Adler, M. and Gutman, A.B. Uridine isomer (5-rikosyluracil) in human urine. Science 130:362, 1959.

6. Drahovsky, D., Winkler, A. and Skoda, J. Urinary excretion of pseudouridine in the course of x-ray therapy. Neoplasma 12:561, 1965.

7. Evans, A.S., Quinn, F.A., Brown, J.S. and Strike, T.A. Effect of ionizing radiation on total protein-bound neutral hexoses in the plasma of mice. Radiation Research 36:128, 1968.

8. Evans, A.S. Effect of ionizing radiations on concentration and distribution of plasma protein-bound neutral hexoses in mice and dogs (abstract). Radiation Research, in press.