

DEPARTMENT OF THE ARMY
OFFICE OF THE SURGEON GENERAL
RESEARCH AND DEVELOPMENT DIVISION
WASHINGTON 25, D. C.



Form Approved
Budget Function No.

APPLICATION FOR RESEARCH CONTRACT - PART 1

DO NOT WRITE IN THIS SPACE

NEW RENEWAL
CONTRACT NUMBER

1. TITLE OF PROJECT

Metabolic changes in Humans following Total Body
Radiation

2. DATE OF BEGINNING
3. DATE OF TERMINATION

3. NAME AND OFFICIAL POSITION OF RESPONSIBLE INVESTIGATOR

Eugene L. Saenger, M.D., Associate Clinical Professor of
Radiology

4. DATE OF COMMENCEMENT
5. DATE OF COMPLETION

3. PRINCIPAL PROFESSIONAL ASSISTANT (S)

George M. Guest, M.D., Professor of Research Pediatrics
Helen K. Berry, M.A., Research Associate, Children's Hospital Research
Foundation
Harold Perry, M.D., Assistant Professor of Radiology

RCC1.950530.002

6. OTHER PROJECTS IN WHICH YOU ARE PARTICIPATING AND SOURCE OF SUPPORT (Other government contracts or funds from civilian sources)

- a) Incidence of Neoplasia in Irradiated Children; supported by National Institutes of Health, P.H.S., C-2973
- b) Preparation of Handbook on Medical Aspects of Radiation Accidents, supported by U.S. Atomic Energy Commission, contract no. AT(30-1)-200

7. NAME AND LOCATION OF INSTITUTION WHERE WORK WILL BE PERFORMED

Departments of Radiology, University of Cincinnati, College of Medicine,
Cincinnati, Ohio

SIGNATURE OF RESPONSIBLE INVESTIGATOR

Eugene L. Saenger

8. APPLICATION APPROVED BY OFFICIAL AUTHORIZED TO SIGN FOR INSTITUTION

NAME PRINTED OR TYPED

Stanley E. Dorst, M.D.

SIGNATURE

Stanley E. Dorst

OFFICIAL TITLE

Dean, College of Medicine

INSTITUTION

University of Cincinnati

9. APPLICATION APPROVED BY HEAD OF DEPARTMENT WHERE WORK IS TO BE PERFORMED

NAME PRINTED OR TYPED

Benjamin Felson, M.D.

SIGNATURE

Benjamin Felson

OFFICIAL TITLE

Professor of Radiology and Head of Department of Radiology

Statement A
Approved for public release;
Distribution unlimited.

DEPARTMENT OF THE ARMY
OFFICE OF THE SURGEON GENERAL
RESEARCH AND DEVELOPMENT DIVISION
WASHINGTON 25, D. C.



Form Approved
Budget Item No.

APPLICATION FOR RESEARCH CONTRACT - PART I

DO NOT WRITE IN THIS SPACE

NEW RENEWAL
CONTRACT NUMBER

1. TITLE OF PROJECT Metabolic changes in Humans following Total Body Radiation	2. DATE OF BEGINNING 1957
3. NAME AND OFFICIAL POSITION OF RESPONSIBLE INVESTIGATOR Eugene L. Saenger, M.D., Associate Clinical Professor of Radiology	4. DATE OF COMPLETION 25 5 1958

5. PRINCIPAL PROFESSIONAL ASSISTANT (S)

George M. Guest, M.D., Professor of Research Pediatrics
Helen K. Berry, M.A., Research Associate, Children's Hospital Research Foundation
Harold Perry, M.D., Assistant Professor of Radiology

6. OTHER PROJECTS IN WHICH YOU ARE PARTICIPATING AND SOURCE OF SUPPORT (Other government contracts or funds from civil service, etc.)

a) Incidence of Neoplasia in Irradiated Children; supported by National Institutes of Health, P.H.S., C-2973

b) Preparation of Handbook on Medical Aspects of Radiation Accidents, 1957, by U.S. Atomic Energy Commission, contract no. AT(30-1)-2200

7. NAME AND LOCATION OF INSTITUTION WHERE WORK WILL BE PERFORMED

Departments of Radiology, University of Cincinnati, College of Medicine, Cincinnati, Ohio

SIGNATURE OF RESPONSIBLE INVESTIGATOR *Eugene L. Saenger*

8. APPLICATION APPROVED BY OFFICIAL AUTHORIZED TO SIGN FOR INSTITUTION

NAME PRINTED OR TYPED
Stanley E. Dorst, M.D.

SIGNATURE
Stanley E. Dorst

OFFICIAL TITLE
Dean, College of Medicine

INSTITUTION
University of Cincinnati

9. APPLICATION APPROVED BY HEAD OF DEPARTMENT WHERE WORK IS TO BE PERFORMED

NAME PRINTED OR TYPED
Benjamin Felson, M.D.

SIGNATURE
Benjamin Felson

OFFICIAL TITLE
Professor of Radiology and Head of Department of Radiology

APPLICATION FOR RESEARCH CONTRACT - PART II

TITLE OF PROJECT		
Metabolic changes in Humans following Total Body Radiation		
FUNDS REQUESTED (One year only)		
REQUIREMENTS	BUDGET	
	REQUESTED (From Office of The Surgeon General)	OTHER SOURCES
1. PERSONNEL (List positions, salaries, and names of professional personnel, if known)		
Technician (fulltime)	\$ 4400.00	-
Technician (fulltime)	4000.00	-
Physicist (part time)	1000.00	Univ. Cinti
Clinician (part time)	2000.00	" "
Statistician (part time)	1000.00	" "
Secretary (part time)	1500.00	" "
total	\$ 13,900.00	
2. EQUIPMENT (Itemize)		
Remodeling of laboratory room	\$900.00	
Densitometer & filters	\$460.00	Ultraviolet lamp \$100.00
Centrifugal chromatograph	250.00	VanSlyke apparatus 350.00
Freeze drying apparatus & Vacuum pump	200.00	chromatographic columns 200.00
Refrigerator	250.00	Colorimeter 450.00
Spray equipment	150.00	
total	\$3310.00	
3. CONSUMABLE SUPPLIES (Itemize)		
Miscellaneous glassware & micropipettes	570.00	
Chemicals, chromatography paper & supplies	1740.00	
Phantoms, etc.	200.00	
	\$2510.00	
4. TRAVEL (State Purpose)		
Conferences and scientific meetings	500.00	
5. SUB-TOTAL		
	\$20,220.00	
6. OVERHEAD (Established by official auditors with concurrence of institution or research agency and contracting officer, and may be based upon percentage of total salaries and wages, or percentage of total cost of the project. Indicate below.)		
Provisional 35% of salaries	4,865.00	
	6685.00	
-----PERCENT OF SALARIES AND WAGES-----PERCENT TOTAL COST		
7. TOTAL BUDGET		
	\$25,085.00	\$25,085.00
8. ESTIMATE OF FUTURE REQUIREMENTS		
<i>(To be filled out only if type of project indicates that it will continue for more than a year)</i>		
FIRST ADDITIONAL YEAR	\$ 21,775.00	
SECOND ADDITIONAL YEAR	\$ 21,775.00	

* Other Sources - from the school, other contracts, other government agencies, foundations, etc.

APPLICATION FOR RESEARCH CONTRACT - PART III

TITLE OF PROJECT **Metabolic Changes in Humans Following Total Body Radiation**

RESEARCH PLAN

(Include background, specific aims, methods of procedure in detail, significance of this research. Use additional pages, if necessary.)

3. Scientific background.

Several reports have described changes in nitrogen metabolism following irradiation (1, 2, 3). All have shown increase in nitrogen excretion following total body irradiation to various laboratory animals. Mefford and Martens (4) have studied amino-aciduria by paper chromatography in rats. Katz and Hasterlik (5), and Hempelmann, Lisco and Hoffman (6) have studied amino-aciduria following radiation in humans by means of paper chromatography. Hempelmann, et al, found amino-aciduria in 3 of their 9 cases.

Katz and Hasterlik reported increases of as high as ten times normal values of total daily amino acid excretion in 4 patients. Quantities of individual amino acids excreted varied from 2 - 20 times normal values. Abnormal values were found as early as 12 hours following exposure, and increased levels persisted for as long as 5 months. No direct quantitative relation to radiation dose could be established.

These findings suggest that amino-aciduria may serve as an indicator of the biological response of humans to irradiation. The reports of amino-aciduria in humans have described the findings in individuals exposed in reactor accidents (5, 6) and no control measurements were possible. The proposed investigation will include studies of amino-acid excretion before and after irradiation both to the whole body and to portions of the body. The urinary excretion of amino acids will be measured both by paper chromatography and total amino acid nitrogen in order to find a relatively simple technique for biological effects of irradiation. Significant increases in total amino acids and individual amino acids will be sought. The amino acid levels will be compared to levels of creatine (10) and urea (11).

These studies will be valuable in the understanding of the mechanisms of amino-aciduria. Preliminary studies by our group have demonstrated that transient amino-aciduria will also occur with extensive surgical procedures and with infection. Studies of 5 patients exposed at the Y-12 accident at Oak Ridge in June 1958 showed elevated excretion of beta aminoisobutyric acid with levels related to the total dose received by the individual.

4. Scientific Scope of the Proposed Research.

Amino-aciduria following irradiation has been reported in humans and animals. The purposes of this investigation is to study this phenomenon to

(3)

APPLICATION FOR RESEARCH CONTRACT - PART III

TITLE OF PROJECT **Metabolic Changes in Humans Following Total Body Radiation**

RESEARCH PLAN

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elucidate some of the mechanisms responsible for amino-aciduria and to determine whether it is a practical biological test of radiation exposure.

The Design of the Proposed Research is as follows:

1. Comparison of techniques for determination of urinary amino acids by the Van Slyke and chromatographic techniques.
2. Determination of excretion of urinary amino acids at various dosages of total body irradiation.
3. A study of the effects of partial versus total body irradiation on urinary amino acid excretion.
4. Studies of the immune mechanisms of humans receiving total body irradiation

Selection of Patients

Patients for total body irradiation will be limited to adult males who have proven metastatic malignancy but are in good nutritional state. The studies will be limited to males because of the variations in amino-aciduria found with the menstrual cycle in women. These patients will be used for parts 1 and 2. In part 3 male patients who are receiving radiation therapy for neoplasms will also be included. Except in special cases as noted below, patients with lymphomas will not be used in these studies. All patients will be hospitalized at the Cincinnati General Hospital. They will have histories, physical examinations, routine hematological and urine studies and other laboratory studies as needed.

Factors for irradiation are as follows:

Westinghouse Quadrocondex constant potential therapy unit 250 KV, 15 Ma, filtration 2.0 mm Cu + 1 mm Al., H.V.L. 2.0 mm Cu. TSD 200 cm. Also total body irradiation will be carried out with Cobalt 60 teletherapy. Measurements will be checked by the Victoreen condenser r-meter and calibrated Bendix dosimeters using masonite and water phantoms. The technique of irradiation will be similar to that of Sinclair & Cole (12).

Urine Studies

Part 1. Comparison of paper Chromatography and Total Amino acid Nitrogen Techniques.

Two groups of 6 adult males will receive 50 r of total body radiation in a single dose. Group A will consist of patients with relatively radio-resistant lesions (e.g.

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stomach, bowel, brain) and Group B of patients with highly radio-sensitive tumors (lymphomas).

Prior to irradiation urines will be collected for several days to serve as controls. All urines will be collected for 24 hour periods. An aliquot from the early morning fasting sample will be refrigerated and the remainder pooled with the 24 hour urine below. The voidings will then be collected, refrigerated, pooled and frozen for the 24-hour periods. All samples will be analyzed in triplicate by both techniques for fasting and pooled values. If the initial fasting specimen provides enough data, the test can be simplified.

Total urinary amino acids will be determined by the method of Van Slyke, MacFayden and Hamilton (7). Two dimensional paper partition chromatography will be carried out according to the methods of Block, Durrum and Zweig (9).

The 24 hour urines will be analyzed for uric acid, urea creatine, creatinine and total nitrogen. An attempt will be made to maintain the patients in approximately the same nitrogen equilibrium.

The values obtained by the two methods will be compared by analysis of variance and by determination of regression coefficients of the values of the Van Slyke method versus those of chromatography. In addition comparison of amino acid excretion in patients with known destruction of large masses of tumor (Group B) can be compared to those in whom such a phenomenon is unlikely (Group A).

Part 2. Determination of Excretion of Urinary Amino Acids at Various Dosages of Total Body Irradiation

Patients to be studied will include those individuals with metastatic neoplasms and also patients who are receiving radiation therapy in an attempt to cure or palliate various neoplasms. Patients in this study will not have had previous irradiation so as to avoid carry-over effects. In the initial selection of patients the group of patients with metastatic neoplasms cannot be selected at random from a normal population for inclusion in the study since normal individuals cannot be given total body radiation.

Selection criteria are as follows:

1. Patient to have proven diagnosis of neoplasm.
2. Patient to have proven evidence of metastasis.
3. Patient to be in satisfactory nutritional state.

(5)

APPLICATION FOR RESEARCH CONTRACT - PART III

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The patients will be selected for three subgroups of total body irradiation by randomization. Subgroup A will serve as controls and will have all procedures carried out except that they will receive sham irradiation only. Subgroup B will receive 25 r and Subgroup C will receive 50 r. Each group will consist of 4 patients. The mean values of total amino acids in the urine of the three groups will be tested for significant differences by analysis of variance. Subgroup A will be tested against Subgroups B and C. Also Subgroup B will be tested against Subgroup C. Further groups will be studied at 100 r and 200 r.

If there is no significant difference between B and C, then additional subgroups will be studied at exposures of 0, 5, 10 and 20 r of total body irradiation. One of the possible reasons that a relation between radiation exposure and amino acid excretion may reach a plateau value below 25 r of total body irradiation. It is for this reason that these additional groups may require study. Patients will be placed in these several groups by the same technique of randomization as described above. Another group of sham irradiated controls will be provided in order not to lose independence of comparisons.

At the conclusion of these studies further analysis of the data will be carried out with calculations based on the integral dose received by each patient. The dosage response relationship will then be determined.

Part 3. Study of the Effects of Partial versus Total Body Irradiation on Urinary Amino Acid Excretion

After suitable base line values are established as described Part 2, further studies will be carried out in order to compare total body irradiation with doses of localized irradiation to give equal integral dosage. For example, if a patient receives total body irradiation with an integral dose of 50,000 gram roentgens, his amino-aciduria will be compared with that of an individual who has received an equal integral dose with all irradiation given to a localized region of the body. One group of patients will receive the dose to the upper abdomen, another group to the chest, another group to the head and neck. These patients will have determinations of urinary amino acids before receiving radiation and will be followed afterwards for a period of one to three years.

The first group to be studied will be those patients who receive localized irradiation to the kidneys and liver. Since irradiation to either of these organs

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may provide a mechanism for amino-aciduria (see Part I) it will be valuable to determine the levels of amino-aciduria following this type of irradiation. It must be recognized that in applying radiation to the human it is not possible to irradiate the liver without irradiating the kidneys and conversely one cannot irradiate the kidneys without irradiating the liver. If the amino-aciduria is of the same order of magnitude as that found with total body irradiation, it will suggest that irradiation of either or both of the organs is responsible for these findings. The second group to be studied are those individuals receiving localized radiation to the chest. If the amino-aciduria approaches levels equal to those found with total body irradiation in comparable integral doses, increased tissue breakdown should be suspected.

From a practical viewpoint these two groups will be studied simultaneously depending upon clinical material available on the Tumor Service of the Cincinnati General Hospital.

Part 4.

In cooperation with Dr. A. J. Luzzio of the Radiobiology Department, U. S. Army Medical Research Laboratory, Ft. Knox, Ky, we shall supply blood serum samples from all patients irradiated as described previously. Samples will be obtained just prior to irradiation, just after irradiation and ten days after irradiation. They will be sent to Dr. Luzzio for his studies.

REFERENCES

1. Brues, A., Nuclear Science Abstracts, 7:7, 1953.
2. Gustafson, G. E., and Koletsky, S., Am. J. Physiol. 171: 319 (1952).
3. White, J., Burr, B. E., and Stander, Argonne National Laboratory Quarterly Report, Feb. Mar. April 1952, 4794.
4. Mefford, R. B., Martens, H. H., Science, 122:829, (1955).
5. Katz, E. J. and Hasterlik, R. S., J. National Cancer Inst., 15:1085 (1955).
6. Hempelmann, L. H., Lisco, H. and Hoffman, J. D., Ann. Int. Med. 36:279 (1952).
7. Van Slyke, D. D., MacFayden, D. A. and Hamilton, P. B. J. Biol. Chem. 150: 251, (1943).
8. Moore, S., and Stein, W. H., J. Biol. Chem. 211: 907 (1954).
9. Block, R. J., Durrum, E. L. and Zweig, G., A Manual of Paper Chromatography and Paper Electrophoresis, Academic Press, Inc., New York 1955.

APPLICATION FOR RESEARCH CONTRACT - PART III

TITLE OF PROJECT

Metabolic Changes in Humans Following Total Body Radiation

RESEARCH PLAN

(Include background, specific aims, methods of procedure in detail, significance of this research. Use additional pages, if necessary.)

10. Anderson, D.R. Effects of Radiation on Creatine Metabolism. Radiation Research 7:300 (1957)
11. Kay, R.E., Early, J.C. and Entenhan, C. Radiation Research 6:98-109 (1957)
12. Sinclair, W.K. and Cole, A., Technique and dosimetry for whole body x-irradiation of patients. USAF Report No. 57-70 Mar. 1957.

APPLICATION FOR RESEARCH CONTRACT - PART IV

BIOGRAPHY

(Biographical sketches required on responsible investigator and principal professional assistants only. If this is a request for renewal utilizing essentially the same personnel as previously, biographical sketches will not be necessary.)

1. NAME	2. ADDRESS	3. AGE
Eugene L. Saenger, M.D.	Radioisotope Laboratory, Cincinnati General Hospital, Cincinnati 29, Ohio	41

4. EDUCATIONAL BACKGROUND (College and/or University)

Harvard College A. B., cum laude Biochemical Sciences 1938
University Cincinnati, College of Medicine, M. D. 1942

5. RESEARCH TRAINING (List of institutions, research director, subject and dates)

Interne General Hospital, Cincinnati 1942-1943
Resident in Radiology, General Hospital 1943-46
Chief Radioisotope Unit, Brooke Army Hospital, FSHT 1954-55
Consultant Surgical Research Unit 1954-55
Director, Radioisotope Laboratory, Univ. of Cincinnati, Coll. of Medicine 1950-
Assistant Professor of Radiology 1949-57
Assoc. Clinical Professor of Radiology 1957-
Consultant Brooke Army Hospital 1956-

6. OTHER INFORMATION BEARING ON QUALIFICATIONS (Hospital appointments, professional societies, specialty board, etc.)

Attending Radiologist, Cincinnati General Hospital;
Radiation Therapist Children's Hospital, Cincinnati, Ohio;
Diplomate, American Board of Radiology, Member
American Roentgen Ray Society, Radiological Society of North America, Health
Physics Society; Alpha Omega Alpha

7. BIBLIOGRAPHY (Do not list more than ten publications)

1. Saenger, E. L., et al, Emergency Measures and precautions in Radium accidents. Jour. Med. Assn. 149:813-815 June 28, 1952.
2. Results of Therapy with Radioactive Iodine-131 in Hyperthyroidism. Ohio State Med. Jour. 50:26-27, Jan 1954. E. L. Saenger, et al.
3. Saenger, E. L., et al, Letterer-Siwe's Disease. Problems in Diagnosis and Treatment. Amer. Jour. Roent. Rad. Therapy & Nucl Med. LXXI, No. 3, March 1954.
4. Protection against radiations from radium, cobalt-60, and cesium-137; National Bureau of Standards Handbook 54, section 9, accidents entailing radiation hazards, Sept. 1954. Saenger, E. L., et al.
5. Saenger, E. L. et al, Carcinoma of the Prostate; Therapy with Radioactive Colloidal gold. U.S. Armed Forces Med. Jour. VII:469 (April 1956).
6. A Method for Monitoring background by means of statistical control chart. The Amer. Jour. Roent., Rad. Therapy & Nuc. Med. LXXV June 1956, Saenger, et al.
7. Saenger, E. L. et al, A method for labeling the lone star tick with radioactive indicator (P-32). Jour. of Economic Entomology 49:393, June 1956.
8. Radiation Hazards in the practice of surgery. The Am. Surgeon, 22:676, 1956.
9. Saenger, E. L. et al, Radium capsules and their assoc. hazards. Am. Jour. Roent. 77:511, March 1957.

APPLICATION FOR RESEARCH CONTRACT - PART IV

BIOGRAPHY

(Biographical sketches required on responsible investigator and principal professional assistants only. If this is a request for renewal utilizing essentially the same personnel as previously, biographical sketches will not be necessary.)

1. NAME Guest, George Martin, M.D.	2. ADDRESS Children's Hospital Research Foundation, Cincinnati 29, Ohio	3. AGE 60
4. EDUCATIONAL BACKGROUND (College and/or University)		
Ohio Wesleyan University, Delaware, Ohio 1916-18		
University of Cincinnati, B.S. 1920		
" " " College of Medicine M.D. 1922		
" " " " " " M.S. 1923		
5. RESEARCH TRAINING (List of institutions, research director, subject and dates)		
Cincinnati General Hospital, Resident in Pathology and Instructor Pathology 1922-1923		
Institut Pasteur, Brussels, Belgium, Fellow of the Commission for Relief in Belgium		
Educational Foundation 1923-1925		
Boston Children's Hospital, Intern in Pediatrics, Resident Bacteriologist and Ass't '25		
in Bacteriology, Harvard Medical School 1926-1927		
University of Cincinnati, College of Medicine, Dept. of Pediatrics: Ass't Prof. 1928-19		
" " " " " " Assoc. Prof. 1935-19		
" " " " " " Prof. of Research Pediatrics 1947-		
6. OTHER INFORMATION BEARING ON QUALIFICATIONS (Hospital appointments, professional societies, specialty board, etc.)		
Am. Pediatric Soc. (Member of Council; Chairman 1953) Alpha Omega Alpha		
Soc. for Pediatric Research (President 1942) Sigma XI		
Am. Soc. of Biological Chemists		
Certified Licentiate of the Am. Board of Pediatrics 1936		
Editorial Board Diabetes		
7. BIBLIOGRAPHY (Do not list more than ten publications)		
1. Guest, G.M. et al, Centrifuge method for the determination of the volume of cells in blood, J. Lab. & Clin. Med. 19:757-767, April 1934.		
2. Guest, G.M., et al, Hematologic methods in detecting nutritional anemia, in Nutrition: The newer diagnostic methods, 17th ann. conf., Milbank Memorial Fund, N. pp. 138-165, 1938.		
3. Guest, G.M. et al, Organic acid-soluble phosphorus compounds of the blood. Physiol. Rev. 21:410-437, July 1941.		
4. Guest, G.M., et al, Diabetic coma: metabolic derangements and principles for corrective therapy, Am.J. Med. 7:630-646, 1949.		
5. Guest, G.M., et al, Urinary excretion of amino-acids during alloxan-induced diabetes in rats, Proc. Soc. Exper. Biol. & Med. 71:410-412, 1949.		
6. Guest, G.M., Diabetic coma, Am.J. Med. 7:630, 1949.		
7. Guest, G.M., et al, Galactosemia, or galactose diabetes, Diabetes 3:330, July 1954		
8. Guest, G.M., et al, Apparatus for continuous recording of insensible weight loss (IWL); clinical applications, Federation Proceedings March 1957, vol 16, 189-190.		