

1. Chairman
 TO: J. R. Come Contract Board. From: Res. Dev. Div.

It is requested that the Contract Board take the necessary action to process the following described contract action in accordance with the provisions of Bulletin OR-O&M-19:

2. Nature of Action Requested

- Selection of New Contractor and Negotiation of Contract.
- Modification of Contract
 No. AT-(10-1)-1038
 Contractor: Southern Research Institute
Birmingham 5, Alabama
- Review and approval of Contract, Sub-contract or Purchase Order.
 Number: _____
 Name: _____
- Other (Explain) _____

3. Nature of Services to be Covered by Contract

Construction Architect-Engineer Other (Explain) Research

4. Funding Amount to be Obligated by this Contract Action \$ 3,252.00

Source of Funds

Approved ORO Financial Plan, _____ Quarter, Fiscal Year 19____
 Project No. _____ or, Activity No. 6130
 Funds to be Obligated: Allotment No. 6-4-91(22) F.Y. 1959 Funds)
 Procurement Directive No. BM-69-95 Dated 6-31-59
 Issuing Office Div of Biology & Medicine

Concurrence in Funding Statement: (signed) OS Miller
 Chief, Budget Branch

5. Project or Activity to be Covered by Contract Action:

Location of Work: _____ Construction Directive No. _____
 Estimated Cost of Work to be Covered by this Contract Action \$ _____
 Schedule: Date Work to Start _____ Estimated Completion Date _____
 Description of Project or Activity:

(If more space is required use separate sheets and attach hereto:)

<p>6. Contract Board Docket No. _____ (To be assigned by Board Secretary)</p>	<p>7. Request Submitted By: (signed) <u>C. S. Shoup</u> Date: <u>Oct 29 1959</u> File: _____ C. S. SHOUP CHIEF, BIOLOGY BRANCH RESEARCH AND DEVELOPMENT DIVISION</p>
<p>8. <u>Complete Description of Services to be Furnished by Contractor:</u></p> <p>Headquarters designated research contract TITLE: "Body Retention of Carbon-14"</p> <p>(If more space is required use separate sheets and attach hereto)</p>	
<p>9. <u>Description of other changes to be covered by Modification:</u></p> <p>Modify Contract to provide for the performance of additional research to be completed not later than September 30, 1960. The AEC will contribute \$3,252. The Contractor will contribute \$5,435, and the estimated unexpended balance is \$1,997.</p> <p>(If more space is required use separate sheets and attach hereto)</p>	
<p>10. <u>Negotiated Contracts.</u> (Show why it appears desirable to negotiate new contract or to negotiate modification to existing contract)</p> <p>Memorandum from J. W. Shilling to S. R. Sapirie dtd. 10-13-59</p> <p>(If more space is required use separate sheets and attach hereto)</p>	
<p>11. <u>Contracts, Subcontracts, or Purchase Orders Submitted for Review and Approval:</u> (Furnish brief description of action in this space and attach pertinent documents)</p> <p>None</p>	
<p>12. <u>Disputes:</u> Attach a statement summarizing the dispute together with pertinent documents and Background Material.</p> <p>None</p>	

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APPENDIX "A"

TITLE XI

This TITLE XI describes the research program and cost estimates agreed upon between the Commission and the Contractor.

1. PROGRAM

a. Scope and Plan of Approach:

The Contractor will continue studies on the retention in the body of radioactive Carbon-14 and the effects upon animal tissues, including the extension of these studies to bacteria. The program of work will be essentially as follows:

1. Biological Effects of Carbon-14 Incorporated into DNA of the Fetus.
 - a. Pregnant rats injected singly with 100 uc of sodium formate-C14 as a precursor of purines and thymine will be used and their progeny studied. Animals will be kept under close daily observation, will be autopsied, and thoroughly studied with regard to pathology. Animals now on hand which have been treated with C14 will be bred to see if there is any gross genetic damage.
2. Experiments with Bacterial Systems.
 - a. E. coli will be highly labeled during growth with a C14 precursor of DNA, the labeled cells will be preserved with suitable control cells on agar for periods of months. From time to time cultures will be made, and the number and nature of bacterial mutations will be studied by the Adelberg technique, by (a) culturing on minimal media, (b) treatment with penicillin to kill growing cells (the parent wild line), (c) treatment with penicillinase to destroy penicillin, (d) addition of enriched medium to promote growth of auzotrophs that did not grow on the minimal medium and hence were not killed by penicillin, and (e) isolation and characterization of the mutants. Isolation and characterization will include isolation of mutants with specific growth requirements. After labeling and storing the bacteria, the DNA of the labeled bacterial will be subjected to the effects of Carbon-14 radiation and transmutation, so it should be possible to study cumulative long-term effects.

3. Experiments with Cells in Tissue Culture.

- a. Mammalian cells in tissue culture will be used for DNA labeling by utilizing C¹⁴ precursors. These cells will be stored on a long-term basis, and thawed cells will be studied in culture and clones will be isolated by the technique of Puck. Mutants judged by morphology, and effects of radiation, will be identified and studied.

2. BUDGET

a. Outline of Cost Estimates:

(1) <u>Salaries and Wages:</u>		\$ 7,850.00
Dr. H. E. Skipper (5% of time)	\$1,000.00	
Research Associates	4,450.00	
Research Assistants	2,100.00	
Animal Keeper	300.00	
(2) <u>Retirement Fund:</u>		290.00
(3) <u>Materials and Supplies:</u>		1,350.00
(4) <u>Overhead (78.9% of Salaries and Wages):</u>		<u>6,194.00</u>
	Total	\$15,684.00

- b. Items of property to be procured or manufactured by the Contractor, or to be furnished by the Government, title to which will vest or remain in the Government (see Article V): None

UNITED STATES ATOMIC ENERGY COMMISSION
WASHINGTON, D. C.

Contract Authorization No. BM-50-25

TO : S. R. Sapirie, Manager
Oak Ridge Operations Office

FROM : C. W. Shilling, M.D., Deputy Director
Division of Biology and Medicine

SUBJECT : FUND AUTHORIZATION AND TRANSMITTAL OF RESEARCH PROPOSAL FOR
CONTRACT NEGOTIATION

REFERENCE : AEC 102/16 APPROVED OCTOBER 7, 1953, AS IMPLEMENTED BY MEMORANDUM
TO MANAGERS, OPERATIONS OFFICES, DATED OCTOBER 23, 1953, JOINTLY
SIGNED BY THE DIRECTORS OF THE DIVISIONS OF RESEARCH AND BIOLOGY
AND MEDICINE.

SYMBOL : BMB:LGA

OCT 13 1959

The research proposal described below has been approved by the
Division of Biology and Medicine, funds are available, and you
are authorized and requested to negotiate a contract in
accordance with the following terms and conditions:

1. Institution: Southern Research Institute
2. Investigator (s): Dr. Howard E. Skipper
3. Title: "Body Retention of Carbon-14"
4. () New Contract, (x) Renewal of Contract No. AE(40-1)1038
5. Duration: 10-1-59 thru 9-30-60
6. AEC Technical Representative: Dr. L. G. Augenstine *A*
7. Funds are authorized for the obligation of this contract
as follows:

<u>Allotment No.</u>	<u>Budget Category</u>	<u>Previous</u>	<u>Amount</u>	
			<u>This Action</u>	<u>Total</u>
<u>06-01-91(24)</u>	<u>6130</u>	<u> </u>	<u>\$8,252</u>	<u>\$8,252</u>
<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>

11-10-59

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- 8. It is suggested that in the best interests of the government the following type contract be negotiated: Lump sum
- 9. It is requested that the title to any capital equipment procured under this contract shall be vested with:
 - (x) the contractor; () the government.
- 10. If radioisotopes are to be used in this research, it is requested that the savings available to the contractor under the Radioisotope Research Support Program (Ref. AEC Manual Chapter 7510) be considered in the negotiation of the amount to be funded under this contract.
- 11. Other comments: This contract was approved at the level requested of \$8,252, plus approximate unexpended funds of \$1,997, or a total of \$10,249.
This is the terminal year for this project.

12. Security Requirements:

In accordance with the provisions of Chapter 3403 of the AEC Manual and the requirements of the Declassification Guide, it has been determined that the following security precautions should be taken in connection with the proposed research contract:

Since there is essentially no chance for the development of restricted data, this project has been placed in Category I as defined in Chapter 3403 of the AEC Manual.

- 13. Reports: (x) Reports are to be required as provided for by "Revised Guide for the Submission of Research Proposals" dated February 8, 1954.

() Special reports instructions are as follows:

- Enclosures:
- () "A" - Proposal, dated OROC has copies
 - (x) "B" - Notification letter, dated Aug 13 1952 Aug 28 1957
 - () "C" - Other correspondence, letters

Distribution:

- | | |
|-------------------------------|--------------------------------------|
| Addressee: Original (w encl.) | Division File: Yellow copy (w encl.) |
| 1st copy (w encl.) | Pink copy (w/o encl.) |
| 2nd copy (w encl.) | |
| | Branch File: White copy (w encl.) |
- Program Analysis Branch:
White copy (w/o encl.)

PROPOSAL FOR RENEWAL OF
CONTRACT NO. AT-(40-1)-1038

On

BODY RETENTION OF CARBON-14

To

ATOMIC ENERGY COMMISSION
BIOLOGY AND MEDICINE DIVISION

Southern Research Institute
Birmingham, Alabama
June 19, 1959
Proposal No. 1269

1141285

REQUEST FOR RENEWAL OF CONTRACT NO. AT-(40-1)-1038

1. Title of the Project: Body Retention of Carbon-14
2. Institute and Department: Southern Research Institute, Biochemistry Division
3. Scientific Background:

Since carbon-14 is produced in some quantity by the explosion of hydrogen bombs, interest in the biological effects of carbon-14 has increased in recent years. Although carbon-14 is a weak beta emitter, its biological hazard derives from its ubiquitous occurrence in biological materials and its long half-life.

Work under the subject grant, which has been in progress for some twelve years, has been concerned with the retention of carbon-14 in animal tissues and the resulting biological effects. Since carbon-14 is such a weak emitter, it might be expected that damage from this isotope would depend largely on the nature of the biological compounds into which it was incorporated. Last year, we began a long-range study which was designed to establish conditions for the maximum hazard for carbon-14 and which was based upon the considerations detailed below. The most sensitive site for possible radiation damage is generally considered to be the chromosomes of which deoxyribonucleic acid (DNA) is the important constituent. Much recent biochemical evidence has accumulated to indicate that DNA is synthesized only in relation to cell division and, once synthesized, is metabolically inert. It therefore seemed probable that, if a carbon-14 labeled precursor were given to pregnant animals there would be extensive incorporation into the DNA of the embryonic tissues and that, in some tissues that are mitotically inactive in the adult animal, carbon-14 thus incorporated might be retained for long periods. Experiments designed to study retention of isotope under these conditions were completed during the year and are summarized in the accompanying report. This study showed that, after birth of the mouse, the carbon-14 that had been incorporated into the DNA of the liver and brain was retained without loss. A separate experiment was then set up to study the biological effects of carbon-14 incorporated under these conditions. This experiment was begun last year and is still in progress (see accompanying progress report).

4. Proposed Research:

Research proposed for the next year represents in part a continuation of long-range experiments already underway and, in addition, some new lines of endeavor, all concerned with the effects of carbon-14 incorporated into DNA.

A. Biological Effects of Carbon-14 Incorporated into DNA of the Fetus. On the basis of the considerations and results outlined in Section 3, a large-scale experiment was set up last year to study the biological effects of carbon-14 incorporated into the fetus. The results obtained to date on this experiment are given in detail in the progress report. To summarize briefly, each of a large group of pregnant animals was given a single injection of 100 μ c of sodium formate-C¹⁴ (a precursor of purines and thymine). The progeny of these animals and those of a control group were then put under daily observation for any differences in life span, incidence of tumors or other disease, weight, and general health that might be attributable to damage from carbon-14. These animals are now one year old and as yet there have been no clear-cut differences between the treated and control groups. Only about sixty of the original five hundred animals (roughly 250 controls and 250 treated) have died during the first year. Since mice on the average do not live much longer than two years, most of the remaining animals should die during the next year and, if the carbon-14 does have any effects, it may be that it is during this period that they will be detected. We propose to keep these animals under the same close daily observation as in the past year, to autopsy each animal for cause of death, and also to do a more thorough pathology where it is indicated by the development of any significant differences between treated and control groups. It is estimated that daily inspection of each animal and thorough autopsies of the four hundred odd animals remaining will consume a considerable portion of the proposed time budgeted for the next year.

In addition, in an attempt to extract the maximum information from this rather lengthy and tedious experiment, we propose to breed the animals now on hand to see if there is any gross genetic damage attributable to the carbon-14. Obviously, as carried out to date, the experiment would detect damage in somatic cells only. How long the progeny of this breeding experiment will be kept will depend on the initial observations on the litters. The actual breeding will be accomplished within the current contract period.

B. Experiments with Bacterial Systems. The experiments described above with intact animals are expensive and tedious, and we would like to do some experiments with simpler systems as an adjunct to the animal experiments. The DNA of bacteria, like that of mammalian cells, is metabolically stable in both growing and resting cells, and therefore the same considerations that were the basis of the work with animals apply also to bacteria. Specifically, we propose to highly label Escherichia coli during growth with a C^{14} -labeled precursor of DNA and to preserve these labeled cells and suitable control cells on agar slants for periods of months. From time to time cultures will be made, and the number and nature of mutations will be studied by use of the Adelberg technique. This procedure consists of the following steps: (a) culturing of the bacteria on minimal medium; (b) treatment with penicillin to kill growing cells (the parent wild line); (c) treatment with penicillinase to destroy penicillin; (d) addition of enriched medium to promote the growth of auxotrophs that did not grow on the minimal medium and hence were not killed by penicillin; and (e) isolation and characterization of the mutants.

Isolation and characterization will be attempted by varying the composition of the medium after step (d) to isolate mutants having a specific growth requirement. Differences in the number and the nature of the mutants produced in carbon-14-containing cultures, as compared to control cultures, should be a measure of the effects of the incorporated isotope.

The use of bacteria for this type of study has many advantages over studies in animals. High incorporation of carbon-14 into amino acids can be attained much more easily and with much less expense. Furthermore, without any experimental effort, after labeling and storing of the bacteria, the DNA of the labeled bacteria will be subjected to the effects of carbon-14 radiation and transmutation: these effects will be cumulative and should become apparent when subcultures are made. Thus, with relatively little effort as compared to that expended in an animal experiment such as that described in the accompanying report, it should be possible to study the cumulative effects of carbon-14 in the DNA over a long period of time.

Many studies have, of course, been made of the effects of isotopes on the production of mutations and chromosome breakages in bacteria and other systems. Some of these studies have been concerned with the genetic effects of phosphorous-32 or tritium incorporated into DNA [For a recent review, see B. S. Strauss, *Radiation Research* **8**, 234 (1958)]. However, we are not aware of experiments with carbon-14 that have been set up with the same orientation and purposes of the present one. It is known, however, from the work of McQuade *et al.* [*Exp. Cell Research* **11**, 249 (1953)] that thymidine- C^{14} incorporated into the DNA of onion roots can

produce chromosome aberrations.

C. Experiments with Cells in Tissue Culture. Mammalian cells in tissue culture also lend themselves to experiments such as those proposed above for E. coli. Like bacteria, cells in tissue culture can be highly labeled with precursors of DNA, and once labeled these cells can be stored in the frozen state for long periods of time. It is not technically feasible to attempt to isolate and characterize mutants in these mammalian cells in the same manner as proposed above for bacteria. Rather we propose to thaw the labeled frozen cells from time to time, prepare a culture and isolate clones by the technique of Puck [Marcus, Cieciura, and Puck, J. Exptl. Med. 104, 615 (1956)].

With mammalian cells, much may be learned by observation of the general morphology of the clones thus produced, and the experiments proposed with mammalian cells will involve a comparison of the number of mutants, as judged by the morphology of clones, produced by C-¹⁴-treated and control groups of cells. Effects of radiation and transmutation on the cell might well be reflected in detectable morphological variations in clones grown from single cells. These studies will be more difficult than those with bacteria and, for this reason, how far they are carried will depend to some extent on the results of initial experiments with bacteria. Several lines of mammalian cells are routinely carried in our laboratory and could be used in this study. We have also had considerable experience with the cloning technique of Puck.

Other experiments related directly to A, B or C above might be undertaken on the basis of results obtained during the year.

5. Scientific Personnel:

Principal Investigators

Howard E. Skipper, up to 5% of time. Ph. D. (Biochemistry).
Assistant Director in charge of Biological Sciences.

Experience: Chemical Warfare Service, 1941-1946: toxicology and mechanisms of action of chemical warfare agents, particularly alkylating agents. Southern Research Institute, 1946 - : screening of agents for anticancer and antiviral activity; mechanism of action of anticancer agents; searches for exploitable biochemical differences between normal and cancer cells; mechanism of resistance to anticancer agents.

Publications: Some eighty publications on cancer chemotherapy and related fields and, in addition, the following publications (supported by the subject grant) on the biological fate of carbon-14:

Skipper, White, and Bryan. Studies on the Hazard Involved in Use of C^{14} . I. Retention of Carbon from Labeled Bicarbonate. J. Biol. Chem. 180, 1187 (1949).

Skipper, White, and Bryan. Body Retention of Carbon-14 from Labeled Sodium Bicarbonate. Science 110, 306 (1949).

Skipper, Bell, and Chapman. Studies on the Hazard Involved in the Use of C^{14} . II. The Effect of a Single Dose of C^{14} -Labeled Sodium Bicarbonate on the Pattern of Deaths from Spontaneous Leukemia in Akm Mice. Cancer Research 10, 362 (1950).

Skipper, Nolan, and Simpson. Studies on the Hazard Involved in Use of C^{14} . III. Long-Term Retention in Bone. J. Biol. Chem. 189, 159 (1951).

Skipper. The Hazard Involved in the Use of Carbon-14. Nucleonics 10, 40 (1952).

Skipper, Simpson, and Bell. Long-Term Radiation of Bone Following Administration of C^{14} Bicarbonate. Proc Soc. Exp. Biol. and Med. 92, 549 (1956).

L. L. Bennett, Jr., up to 10% of time, Ph. D. (Organic Chemistry). Head, Biochemistry Division.

Experience: Instructor in chemistry, University of Georgia, 1943-1944. Southern Research Institute, 1948 - : synthesis of C^{14} -labeled compounds; searches for biochemical differences between normal and cancer cells; mechanism of drug action; chemotherapy, nucleic acid metabolism.

Publications: No publications directly relating to radiation hazard. Ten publications on synthesis of anticancer agents and C^{14} -labeled compounds and on nucleic acid metabolism of cancer cells.

Other Scientific Personnel

Linda Simpson, 25% of time, B. S. Biophysicist,
Biochemistry Division.

Experience: Southern Research Institute, 1948 - :
low-level carbon-14 assay in gas phase; construction and standardization
of gas phase proportional counter for carbon-14 and tritium.

Publications: A Simplified Procedure for Proportional
Counting of C¹⁴-Labeled Carbon Dioxide. International J.
of Applied Radiation and Isotopes 3, 172 (1958).

Co-author of papers on hazards in the use of carbon-14
(see publications of Skipper above).

Daniel Farnell, up to 15% of time. DVM, Chemotherapy
Division.

Experience: Southern Research Institute, 1957 - .
screening of compounds against tumors and leukemias; animal care.

In addition to the above persons who will be primarily
responsible for this project, the following will be available for con-
sultation on the microbiological aspects of the proposed work:

Frank M. Schabel, Ph. D. (Bacteriology and Parasitology).
Head, Chemotherapy Division.

Experience: U. S. Army, Camp Detrick, 1943-46,
Research Associate, University of Chicago, 1946-47; virologist,
Health Department, Chicago, 1947-50; microbiologist, Baptist Hospitals,
Birmingham, 1950-51; Southern Research Institute, 1951 - .
Chemotherapy, serology, neurotropic virus diseases, microbiological
techniques.

Numerous publications in the fields of virology and
bacteriology.

Robert F. Pittillo, Ph. D. (Bacteriology), Senior Scientist,
Chemotherapy Division.

Experience: Parke, Davis and Company, 1955-1959.
Southern Research Institute, 1959 - . Mode of action of antibiotics;
assay of antibiotics; microbial metabolism.

6. Other Personnel:

One assistant bacteriologist, about 25% time.
One assistant biologist, about 25% time.

7. Other Financial Assistance:

The salaries of scientific personnel of this institution are paid out of industrial, federal, and institutional research contracts. Charges to each project are made on the basis of time spent on the project. We have no other contracts concerned with the biological effects of carbon-14.

8. Materials, Equipment, and Facilities:

Completely equipped animal rooms, biochemical laboratories, tracer equipment, and bacteriological and tissue culture laboratories. No additional major equipment is needed. The amount budgeted is an estimate based on past experience.

9. Travel:

No travel allowance is requested.

10. Budget

Salaries:	
Senior biochemist, up to 5% time	\$ 1,000
Senior biochemist, up to 10% time	1,300
Veterinarian, 10-15% time (for gross autopsies)	800
Biophysicist, 25% time	1,600
Assistant bacteriologist, 25% time	1,100
Assistant biologist, 25% time	1,000
Pathologist for thorough autopsy (whether or not needed will depend on results yet to be obtained)	750
Animal keeper, part time	<u>300</u>
Total salaries	\$ 7,850
Contribution to employees' retirement fund	290
Materials and supplies:	
Feed and antibiotics for maintenance of mice	300
Expendable glassware and chemicals	400
Radioactive compounds	300
Components of tissue culture media	250
Miscellaneous (shop services, etc.)	<u>100</u>
Total materials and supplies	<u>1,350</u>
Subtotal	9,490
Overhead (8% of total)	759
SRI contribution	<u>5,435</u>
Total Budget	\$15,684

11. Amount Requested \$10,249

Southern Research Institute's usual research contracts bear an overhead of 100% of scientific salaries. You will note, under the budget category, that we have computed overhead differential at the rate of 78.9% of salaries and wages, including vacation and sick leave. This figure is

based on current rate experience in 1959. The difference between 8% of direct costs and the rate of 78.9% indicates the extent of Southern Research Institute's support of the work outlined in this proposal.

12. Statement of Current Expenditures

(a) As of May 31, 1959, the total expenditures of the amount budgeted for the period 10/1/58 to 9/30/59 were:

AEC participation	\$ 5,020.26
SRI participation	<u>2,811.24</u>
	\$ 7,831.50

(b) Estimate of costs for the remainder of the contract is:

AEC participation	\$ 2,983.00
SRI participation	<u>1,527.76</u>
	\$ 4,510.76

13. Residual Funds

It is anticipated that \$1,997 in present contract funds will be remaining at the end of the contract period.

Birmingham, Alabama
 June 19, 1959
 Proposal No. 1269
 (6:10) llb:mkh, mw

TO : Addressees Listed Below
 FROM : John R. Moore, Director, Contract Division
 SUBJECT: CONTRACTUAL DOCUMENTS FOR REVIEW, COMMENTS AND INITIALING

DATE: October 22, 1958

The document(s) listed below are forwarded for your review, comments and initials. Upon completion of your review, please attach your comments, if any, and forward to next in turn. Expeditious handling of this matter will be appreciated.

Modification 8 to Contract AT-(40-1)-1038 with Southern Research Institute

<u>Addressees:</u>	<u>Division:</u>	<u>Initials</u>	<u>Date:</u>	<u>Remarks:</u>
1. R. G. Humphreys	Contract	RSK	10/27/58	
2. A. B. Miller	Budget	ARM	10/22/58	06-91-91 (24) Funds
3. C. S. Shoup	Res. & Dev.	QSK	10/22/58	
4. L. D. MacKay	Finance	RSK	10/24/58	
5.				
6.				

RETURN TO: Alice Brown Contract Division Tel.: 4719

1. TO: J. R. Moore Chairman Contract Board From: Res. and Dev. Div.

It is requested that the Contract Board take the necessary action to process the following described contract action in accordance with the provisions of Bulletin OR-O&M-19:

2. Nature of Action Requested

- Selection of New Contractor and Negotiation of Contract. Modification of Contract No. AT-(40-1)-1038 Contractor: Southern Research Institute Birmingham 5, Alabama. Review and approval of Contract, Sub-contract or Purchase Order. Other (Explain)

3. Nature of Services to be Covered by Contract

Construction Architect-Engineer Other (Explain) Research

4. Funding Amount to be Obligated by this Contract Action \$ 8,323.00

Source of Funds

Approved ORO Financial Plan, Quarter, Fiscal Year 19 Project No. or, Activity No. 6330 Funds to be Obligated: Allotment No. 26-7-71(24) F.Y. 1959 Funds Procurement Directive No. 67M-57-136 Dated 9/30-58 Issuing Office Div. of Biology & Medicine

Concurrence in Funding Statement: (signed) [Signature] Chief, Budget Branch

5. Project or Activity to be Covered by Contract Action:

Location of Work: Construction Directive No. Estimated Cost of Work to be Covered by this Contract Action \$ Schedule: Date Work to Start Estimated Completion Date Description of Project or Activity:

(If more space is required use separate sheets and attach hereto)

<p>6. <u>Contract Board Docket</u> No. _____ (To be assigned by Board Secretary)</p>	<p>7. <u>Request Submitted By: (signed)</u> Date: <u>7-9-1958</u> Title: <u>C. S. Shoup</u> C. S. SHOUP CHIEF, BIOLOGY BRANCH RESEARCH AND DEVELOPMENT DIVISION</p>
<p>8. <u>Complete Description of Services to be Furnished by Contractor:</u> Headquarters designated research contract TITLE: "Body Retention of Carbon-14" (If more space is required use separate sheets and attach hereto:)</p>	
<p>9. <u>Description of other changes to be covered by Modification:</u> Modify contract to provide for the performance of additional research to be completed not later than September 30, 1959, with new funds in the amount of \$8,323. (If more space is required use separate sheets and attach hereto:)</p>	
<p>10. <u>Negotiated Contracts.</u> (Show why it appears desirable to negotiate new contract or to negotiate modification to existing contract) Memorandum from C. W. Shilling to S. R. Sapirie, dated September 30, 1958. (If more space is required use separate sheets and attach hereto:)</p>	
<p>11. <u>Contracts, Subcontracts, or Purchase Orders Submitted for Review and Approval:</u> (Furnish brief description of action in this space and attach pertinent documents) None</p>	
<p>12. <u>Disputes:</u> Attach a statement summarizing the dispute together with pertinent documents and Background Material. None</p>	

100?

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Budget for Contract No. AT-(40-1)-1038
10-1-58 - 9-30-59

(1) <u>Salaries and Wages:</u>		\$ 7,055.00
Dr. H. E. Skipper (5% of time)	\$1,075.00	
Research Associates	4,000.00	
Assistant Chemist	1,980.00	
(2) <u>Retirement Fund:</u>		204.00
(3) <u>Materials and Supplies:</u>		2,000.00
(4) <u>Overhead (72% of salaries and wages):</u>		5,080.00
		<hr/>
	Total	\$14,339.00

The AEC's contribution to the above budget will be \$8,323; the Contractor's contribution will be \$4,339; and \$1,677 is the estimated unexpended balance.

SOUTHERN RESEARCH INSTITUTE
BIRMINGHAM, ALABAMA

Contract No. AT-(40-1)-1038

Dr. Howard E. Skipper
Project Leader.

BODY RETENTION OF CARBON-14

Resume'

The Contractor will continue work on the body retention and incorporation of Carbon-14 into the mammalian fetus, utilizing mice, in order to study body burden, distribution, and the incorporation of C14-labeled formate, in relation to production of abnormalities or lethality. Evaluation of radiation effects from Carbon-14 will include (a) gross abnormalities in young, (b) life-span information, and (c) assay of tissues for the carbon-14 content, and if possible the isolation of nucleic acid purines to determine carb n-14 incorporation in nucleic acid.

C. S. Shoup

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UNITED STATES ATOMIC ENERGY COMMISSION
WASHINGTON, D. C.,

Contract Authorization No. EM-59-136

TO : S. R. Sapiro, Manager
Oak Ridge Operations Office SEP 30 1958

FROM : C. W. Shilling, M.D., Deputy Director
Division of Biology and Medicine *C. W. Shilling*

SUBJECT : FUND AUTHORIZATION AND TRANSMITTAL OF RESEARCH PROPOSAL FOR
CONTRACT NEGOTIATION

REFERENCE : AEC 102/16 APPROVED OCTOBER 7, 1953, AS IMPLEMENTED BY MEMORANDUM
TO MANAGERS, OPERATIONS-OFFICES, DATED OCTOBER 23, 1953, JOINTLY
SIGNED BY THE DIRECTORS OF THE DIVISIONS OF RESEARCH AND BIOLOGY
AND MEDICINE.

SYMBOL : **BMB:JLL**

The research proposal described below has been approved by the
Division of Biology and Medicine, funds are available, and you
are authorized and requested to negotiate a contract in
accordance with the following terms and conditions:

- ✓ 1. Institution: Southern Research Institute
- ✓ 2. Investigator (s): Dr. Howard E. Skipper
- ✓ 3. Title: "Body Retention of Carbon-14"
- ✓ 4. () New Contract, (x) Renewal of Contract No. AT(40-1)1038
- x 5. Duration: 10-1-58 thru 9-30-59
- 6. AEC Technical Representative: Dr. *James L. Liverman*
- 7. Funds are authorized for the obligation of this contract
as follows:

<u>Allotment No.</u>	<u>Budget Category</u>	<u>Amount</u>	
		<u>Previous</u>	<u>This Action</u>
<u>Total</u>			
06-91-91(24)	6330		\$10,000
			\$10,000

H 9579
OCT 2 - 1958

8. It is suggested that in the best interests of the government the following type contract be negotiated: Lump sum
9. It is requested that the title to any capital equipment procured under this contract shall be vested with:
- (x) the contractor; () the government.
10. If radioisotopes are to be used in this research, it is requested that the savings available to the contractor under the Radioisotope Research Support Program (Ref. AEC Manual Chapter 7510) be considered in the negotiation of the amount to be funded under this contract.
11. Other comments: None

12. Security Requirements:

In accordance with the provisions of Chapter 3403 of the AEC Manual and the requirements of the Declassification Guide, it has been determined that the following security precautions should be taken in connection with the proposed research contract: Since there is essentially no chance for the development of restricted data, this project has been placed in Category I as defined in Chapter 3403 of the AEC Manual.

13. Reports: (x) Reports are to be required as provided for by "Revised Guide for the Submission of Research Proposals" dated February 8, 1954.

() Special reports instructions are as follows:

Enclosures: (x) "A" - Proposal, dated June 30, 1958
(x) "B" - Notification letter, dated SEP 30 1958
() "C" - Other correspondence, _____ letters

Distribution:

Addressee: Original (w encl.) Division File: Yellow copy (w encl.)
1st copy (w encl.) Pink copy (w/o encl.)
2nd copy (w encl.)

Branch File: White copy (w encl.)

Program Analysis Branch:
White copy (w/o encl.)

RECEIVED

4V029 PB FAX BIRMINGHAM ALA OCT 7 418PMC

US MISSION
ATOMIC ENERGY
COMMITTEE

909

REFERENCE YOUR WIRE OF OCTOBER 7 REGARDING RENEWAL OF
CONTRACT AT-48-1/-1938 TO SEPTEMBER 30 1-5 THE
\$10000.00 REQUESTED SHOULD BE REDUCED BY THE AMOUNT
AS INDICATED ON OUR PROPOSAL

SENT
BY
DATE

ANDREW HYPER JR SOUTHERN RESEARCH INSTITUTE

AT-48-1/-1938

RECEIVED
OCT 8 8:52

OCT 8 - 1958

LOCKHIDE OBSERVATORY
RECEIVED

H 9790

1111304

PROPOSAL FOR RENEWAL OF
CONTRACT NO. AT-(40-1)-1038

On

BODY RETENTION OF CARBON-14

To

ATOMIC ENERGY COMMISSION
RESEARCH AND DEVELOPMENT DIVISION

Southern Research Institute
Birmingham, Alabama
June 24, 1958
Proposal No. 1021

H-6441
JUNE 30, 1958

1111305

REQUEST FOR RENEWAL OF CONTRACT NO. AT-(40-1)-1038

1. Title of the Project: Body Retention of Carbon-14
2. Institution and Department: Southern Research Institute, Biochemistry Division
3. Scientific background

In past years this program has been concerned with the long-term retention of carbon-14 in animal tissues after administration of various C^{14} -labeled organic compounds. Early work dealt primarily with hazards resulting from inhalation or ingestion of barium carbonate- C^{14} or carbon dioxide- C^{14} , with particular attention to long-term retention in bone. More recently, funds from this project were used to support our program on uptake of nucleic acid precursors by animal tissues—a study which provided data for the calculation of radiation to chromosomes resulting from the uptake of a number of labeled compounds.

In the last two years these studies have been given a somewhat different direction. This program has partially supported studies on renewal of nucleic acid purines in rapidly dividing cells. The results of these studies, and those of other investigators working in this same area, have led to the concept of "metabolic stability" or "conservation" of DNA in rapidly dividing cells (bacteria, tissue culture, tumors, embryos). A more detailed account of this concept is given in the accompanying progress report. As applied to the problem of hazards of carbon-14, these results suggested that the maximum conceivable hazard possible with carbon-14 might result from the administration to pregnant animals of a C^{14} -labeled compound that would label nucleic acids. This precursor would be taken up extensively by the fetal tissues and "conserved" in the nucleic acids during fetal growth and also during the life of the animal after birth. Thus, carbon-14 would be introduced at an early stage of fetal development into nucleic acids with the result that chromosomes of the animal would be subjected to the radiation from the incorporated carbon-14 for extended periods.

4. Proposed Research

During the past year, effort on this project has been directed to studies of renewal of nucleic acids in embryonic tissue and retention of carbon-14 in nucleic acids of tissues after birth. These experiments, which are described fully in the accompanying progress report, were undertaken as a preliminary to a full-scale testing of the biological effects of

1111306

carbon-14 incorporated into the fetus. This full-scale experiment has now been initiated. For this purpose, one hundred female Swiss mice were mated for 14 days, after which fifty were injected with 100 μ c each of sodium formate-C¹⁴ and fifty with inactive sodium formate. The litters are being observed for deaths or abnormalities that could be ascribed to radiation. There are more than 200 offspring in each group, so that the results should be meaningful.

Experiments of this type, of necessity, must be carried out over a long period of time and the research proposed for the next year is primarily the completion and evaluation of this experiment. The evaluation of possible radiation effects will be made by the following types of studies.

(a) All young will be examined for any obvious gross abnormalities. As the litters approach one month of age, each animal will be caged separately and observations made until death. Since each animal must be observed over an extended period of time, during which infections should be avoided, all animals are maintained on sulfa drugs at appropriate intervals and are caged in a locked room to which only a few people have access.

(b) Careful life-span data will be recorded for both control and treated groups. At death, each animal will be given a gross autopsy and carcasses will be preserved in 10% formalin for more detailed pathology later if it should prove desirable.

(c) Starting at one month after birth, animals will be selected at random from the treated groups and a number of tissues will be assayed for total carbon-14 by gas phase counting methods. If activity is sufficiently high, nucleic acid purines will also be isolated and assayed to determine if the activity is all present in the nucleic acids.

When some of the results on this experiment have become available, other experiments may be indicated. Depending on what the results show, further studies may be undertaken, either with other precursors or at different levels of the same precursor. Tritiated thymidine is now available with high specific activity and, for this type of study, this compound would offer the advantage that it would be incorporated specifically into DNA. In this regard, it is interesting to note that evidence has recently been published [Painter, Drew, and Hughes, *Science* 127, 1244 (1958)] that HeLa cells, grown in tissue culture on a medium containing tritiated thymidine, were inhibited in growth as compared to cells grown with inactive thymidine.

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5. Scientific Personnel

Principal Investigator

Howard E. Skipper, up to 5% of time, Ph.D. (Biochemistry).
Assistant Director in charge of Biological Sciences.

Experience: Chemical Warfare Service, 1941-1946: toxicology and mechanisms of action of chemical warfare agents, particularly alkylating agents. Southern Research Institute, 1946- : screening of agents for anticancer and antiviral activity; mechanism of action of anticancer agents; searches for exploitable biochemical differences between normal and cancer cells; mechanism of resistance to anticancer agents.

Publications: Some eighty publications on cancer chemotherapy and related fields and, in addition, the following publications (supported by the subject grant) on hazards involved in use of C^{14} :

Skipper, White, and Bryan. Studies on the Hazard Involved in Use of C^{14} . I. Retention of Carbon from Labeled Bicarbonate. J. Biol. Chem. 180, 1187 (1949).

Skipper, White, and Bryan. Body Retention of Carbon-14 from Labeled Sodium Bicarbonate. Science 110, 306 (1949).

Skipper, Bell, and Chapman. Studies on the Hazard Involved in the Use of C^{14} . II. The Effect of a Single Dose of C^{14} -Labeled Sodium Bicarbonate on the Pattern of Deaths from Spontaneous Leukemia in Akm Mice. Cancer Research 10, 362 (1950).

Skipper, Nolan, and Simpson. Studies on the Hazard Involved in Use of C^{14} . III. Long-Term Retention in Bone. J. Biol. Chem. 189, 159 (1951).

Skipper. The Hazard Involved in the Use of Carbon-14. Nucleonics 10, 40 (1952).

Skipper, Simpson, and Bell. Long-Term Radiation of Bone Following Administration of C^{14} -Bicarbonate. Proc. Soc. Exp. Biol. and Med. 92, 549 (1956).

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Other Scientific Personnel

L. L. Bennett, Jr., up to 10% of time, Ph.D. (Organic Chemistry). Head, Biochemistry Division.

Experience: Instructor in chemistry, University of Georgia, 1943-1944. Southern Research Institute, 1948- : synthesis of C^{14} -labeled compounds; searches for biochemical differences between normal and cancer cells; mechanism of drug action; chemotherapy.

Publications: No publications directly relating to radiation hazard. Ten publications on synthesis of anticancer agents and C^{14} -labeled compounds and on nucleic acid metabolism.

Daniel Farnell, up to 15% of time. DVM, Chemotherapy Division.

Experience: Southern Research Institute, 1957- : screening of compounds against tumors and leukemias; animal care.

Linda Simpson, 25% of time, B.S. Biophysicist, Biochemistry Division.

Experience: Southern Research Institute, 1948- : low-level C^{14} assay in gas phase; construction and standardization of gas phase proportional counter for carbon-14 and tritium.

Publications: A Simplified Procedure for Proportional Counting of C^{14} -Labeled Carbon Dioxide. International J. of Applied Radiation and Isotopes 3, 172 (1958).

Co-author of papers on hazards in the use of carbon-14 (see publications of Skipper above.)

6. Other Personnel

One assistant chemist, 50% of time.

7. Other Financial Assistance

The salaries of scientific personnel of this institution are paid out of industrial, federal, and institutional research contracts. Charges to each project are made on the basis of time spent on the project.

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We have no other contracts concerned with the hazard from carbon-14; however, we are carrying on other programs on nucleic acid metabolism, and these programs have supported partially the studies on nucleic acid renewal, which forms the background for the proposed research.

8. Materials, Equipment, and Facilities

Completely equipped animal rooms, biochemical laboratories, and tracer laboratories. No additional major equipment is needed. As for C^{14} -labeled compounds, the amount indicated on the budget is an estimate and may be adjusted on the basis of results as they accumulate.

9. Travel

No travel allowance is requested.

10. Budget

Salaries:

Biochemist, up to 5% time	\$1,075.
Biochemist, up to 10% time	1,400.
Veterinarian, approximately 15% time	1,000.
Biophysicist, 25% time	1,600.
Assistant chemist, 50% time	<u>1,980.</u>
Total salaries	7,055.

Contribution to employees' retirement fund	204.
--------------------------------------------	------

Materials and supplies:

Animals, feed, antibiotics, etc.	\$ 500.	
Expendable glassware and chemicals	200.	
Radioactive compounds (if needed)	300.	
Animal cages	<u>1,000.</u>	2,000.

Overhead (8% of total)	741.
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SRI contribution	<u>4,339.</u>
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Total Budget	\$14,339.
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11. <u>Amount Requested</u>	\$ 10,000.
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Southern Research Institute's usual research contracts bear an overhead of 100% of scientific salaries. You will note, under the budget category, that we have computed overhead differential at the rate of 72% of salaries and wages, including vacation and sick leave pay. This figure is

based on current rate experience in 1958. The difference between 8% of direct costs and the rate of 72% indicates the extent of Southern Research Institute's support of the work outlined in the enclosed proposal.

12. Statement of Current Expenditures

(a) As of May 31, 1958, the total expenditures of the amount budgeted for the period 10/1/57 to 9/30/58 were:

AEC participation	\$ 5,628.17
SRI participation	<u>2,496.27</u>
	\$ 8,124.44

(b) Estimate of costs for the remainder of the contract is:

AEC participation	\$4,371.83
SRI participation	<u>2,506.73</u>
	\$ 6,878.56

(c) Estimate of funds which will be available for financing the project during the proposed period of performance:

\$ 1,677.00

13. Residual Funds

It is anticipated that all funds in present contract will be expended.

Birmingham, Alabama
 June 26, 1958
 Proposal No. 1021
 (6:10) llb:nb, mw

1111311

TO : Addressees Listed Below

DATE: October 25, 1957

FROM : John R. Moore, Director, Contract Division

SUBJECT: CONTRACTUAL DOCUMENTS FOR REVIEW, COMMENTS AND INITIALING

The document(s) listed below are forwarded for your review, comments and initials. Upon completion of your review, please attach your comments, if any, and forward to next in turn. Expeditious handling of this matter will be appreciated.

Modification 7 to Contract AF-(40-1)-1038 with Southern Research Institute

<u>Addressees:</u>	<u>Divisions:</u>	<u>Ints:</u>	<u>Date:</u>	<u>Remarks:</u>
1. E. G. Humberies	Contract	ROB	10/25	
2. A. E. Miller	Budget	acm	10/29	
3. C. S. Shoup	Res. & Dev.	JRK OS	10-31 10-31	
4. I. D. MacKay	Finance	BH OS	11	
5.				
6.				
<u>RETURN TO:</u>				
Alice Brown		Contract Division		Tel.: 716

Cont
10
11-6-57

Office Memorandum • UNITED STATES GOVERNMENT

TO : J. W. Gould, Jr., Assistant General Counsel

DATE: October 17, 1957

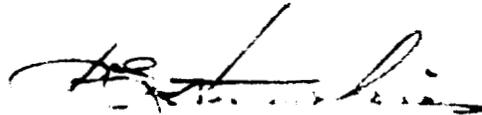
FROM : John R. Moore, Director, Contract Division

SUBJECT: REQUEST FOR MODIFICATION OF CONTRACT AT-(40-1)-1038 WITH
SOUTHERN RESEARCH INSTITUTE, BIRMINGHAM 5, ALABAMA

SYMBOL: ACD:DS

Please prepare an appropriate modification to subject contract to extend the period to September 30, 1958, with new funds in the amount of \$10,000.00.

Request for Contract Action from Research and Development Division is enclosed for your use.



John R. Moore

Enclosures:

1. Request for Cont. Action
2. Budget breakdown
3. Program resume
4. Memo for Wash. Div. of Biology & Med., dtd 10-2-57
5. Request for Renewal

CC: Arthur Schoen
L. L. Mackay
Alice Brown, w/ copy each 1, 2 & 4

1. Chairman
 TO: J. R. Moore Contract Board. From: Research and Dev. Division

It is requested that the Contract Board take the necessary action to process the following described contract action in accordance with the provisions of Bulletin OR-O&M-19:

2. Nature of Action Requested

Selection of New Contractor and Negotiation of Contract.

Modification of Contract No. AT-(110-1)-1038

Contractor: Southern Research Institute
Birmingham 5, Alabama

Review and approval of Contract, Sub-contract or Purchase Order.

Other (Explain) _____

Number: _____

Name: _____

3. Nature of Services to be Covered by Contract

Construction

Architect-Engineer

Other

(Explain) Research

4. Funding

Amount to be Obligated by this Contract Action \$ 10,000

Source of Funds

Approved ORO Financial Plan, _____ Quarter, Fiscal Year 19__

Project No. _____ or, Activity No. 6330

Funds to be Obligated: Allotment No. 06-81-91(24) Y. 1958 Funds)

Procurement Directive No. B7M-58-148 Dated 10-2-57

Issuing Office Division of Biology & Medicine

Concurrence in Funding Statement: (signed) _____

A. E. Miller
 Chief, Budget Branch

5. Project or Activity to be Covered by Contract Action:

Location of Work: _____ Construction Directive No. _____

Estimated Cost of Work to be Covered by this Contract Action \$ _____

Schedule: Date Work to Start _____ Estimated Completion Date _____

Description of Project or Activity: _____

(If more space is required use separate sheets and attach hereto!)

<p>6. Contract Board Docket No. _____ (To be assigned by Board Secretary)</p>	<p>7. Request Submitted By: (signed) Date: <u>OCT 7 1957</u> Title: <u>Arthur Shoup</u> <i>acting</i> C. S. SHOUP CHIEF, BIOLOGY BRANCH RESEARCH AND DEVELOPMENT DIVISION</p>
<p>OK H 10/10</p>	<p>8. Complete Description of Services to be Furnished by Contractor: Headquarters designated research contract. Title: Body Retention of Carbon-14 (If more space is required use separate sheets and attach hereto:)</p>
	<p>9. Description of other changes to be covered by Modification: Modify contract to extend the period to September 30, 1958, with new funds in the amount of \$10,000. (If more space is required use separate sheets and attach hereto:)</p>
	<p>10. Negotiated Contracts. (Show why it appears desirable to negotiate new contract or to negotiate modification to existing contract) Memorandum from C. W. Shilline to S. E. Sepirie dated October 2, 1957 (If more space is required use separate sheets and attach hereto:)</p>
	<p>11. Contracts, Subcontracts, or Purchase Orders Submitted for Review and Approval: (Furnish brief descrip- tion of action in this space and attach pertinent documents) None</p>
<p>12. Disputes: Attach a statement summarizing the dispute together with pertinent documents and Background Material. None</p>	

BUDGET FOR CONTRACT NO. P-(10-1)-1038

PERIOD 10-1-57 - 9-30-58

(1) <u>Salaries and Wages:</u>	\$7,475.00
Dr. H. E. Skipper (10% of time)	\$1,980.00
Research Associates	3,695.00
Assistant Chemist	1,800.00
(2) <u>Retirement Fund:</u>	285.00
(3) <u>Materials and Supplies:</u>	1,500.00
(4) <u>Overhead (76.83% of Salaries and Wages):</u>	<u>5,743.00</u>
TOTAL	\$15,083.00

The NCO's contribution to the above budget will be \$10,000.

SOUTHERN RESEARCH INSTITUTE
BIRMINGHAM, ALABAMA

CONTRACT NO. AT-(40-1)-1038

DR. HOWARD E. SKIPPER

BODY RETENTION OF CARBON-14

Resume'

The Contractor will continue ~~this~~ program, ~~for the next period of performance,~~ extending from the original purpose of determine of Carbon-14 binding and biological hazard of C-14 to studies upon (1) conservation of the nucleic acids in embryonic tissues, studying mice which have received C-14-labeled precursors of purines, (2) studies of embryonic tissues ~~modifications~~ to determine binding of C-14 in the nucleic acids, followed by life period studies, (3) life-span studies to determine minimal level of C-14 causing demonstrable changes and possible long-range development of leukemia, and (4) other appropriate work to evaluate differences in behavior of the adult to C-14 compared with animals which received the radioactive isotope in compounds administered during foetal stages. Special emphasis in general will be on the hazard of C-14 when incorporated into the animal during early stages of development.

U. S. Shoup

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UNITED STATES ATOMIC ENERGY COMMISSION
WASHINGTON, D. C.

Contract Authorization No. EM-58-148

OCT 2 1957

TO : S. R. Sapirie, Manager
Oak Ridge Operations Office

FROM : C. W. Shilling, M. D., Deputy Director
Division of Biology and Medicine, Washington

SUBJECT : FUND AUTHORIZATION AND TRANSMITTAL OF RESEARCH PROPOSAL FOR
CONTRACT NEGOTIATION

REFERENCE : AEC 102/16 APPROVED OCTOBER 7, 1953, AS IMPLEMENTED BY MEMORANDUM
TO MANAGERS, OPERATIONS OFFICES, DATED OCTOBER 23, 1953, JOINTLY
SIGNED BY THE DIRECTORS OF THE DIVISIONS OF RESEARCH AND BIOLOGY
AND MEDICINE.

SYMBOL : **EM:JKT**

The research proposal described below has been approved by the
Division of Biology and Medicine, funds are available, and you
are authorized and requested to negotiate a contract in
accordance with the following terms and conditions:

- ✓ 1. Institution: Southern Research Institute
- ✓ 2. Investigator (s): Dr. Howard E. Skipper
- ✓ 3. Title: Body Retention of Carbon-14

4. () New Contract, (x) Renewal of Contract No. AT(40-1)1038

✓ 5. Duration: October 1, 1957 thru September 30, 1958

6. AEC Technical Representative: Dr. John R. Totter *JRT*

7. Funds are authorized for the obligation of this contract
as follows:

Allotment No.	Budget Category	Amount		Total
		Previous	This Action	
06-81-91(24)	6330		\$10,000	\$10,000

G 8751
OCT 4 1957

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REQUEST FOR RENEWAL OF CONTRACT

NO. AT-(40-1)-1038

On

BODY RETENTION OF CARBON-14

To

ATOMIC ENERGY COMMISSION

DIVISION OF BIOLOGY AND MEDICINE

Southern Research Institute
Birmingham, Alabama
June 28, 1957

1111320

REQUEST FOR RENEWAL OF CONTRACT NO. AT-(40-1)-1038

1. Title of the Project: Body Retention of Carbon-14
2. Institution and Department: Southern Research Institute, Biochemistry Division
3. Scientific Background: -

This program, which has been in progress for nine years, had as its original purpose a study of the retention of carbon-14 in animal tissues, particularly bone, after ingestion or inhalation of $C^{14}O_2$ or C^{14} -labeled organic compounds. The emphasis of these early studies was on the possible hazards involved in the use of carbon-14 in the chemical and biochemical laboratory. The results of these studies indicated that the biological half-life of carbon-14 ingested as $C^{14}O_2$, $BaC^{14}O_3$, or $NaHC^{14}O_3$ was such that there was little hazard in its use unless the doses were massive (in excess of 50 mc man-equivalent). In the last two years of the program, these studies were extended and calculations were made of the level of radiation to which the chromosomes of various tissues were subjected following the administration of a number of precursors of nucleic acids: formic acid, glycine, purines, and purine nucleosides and nucleotides. This phase of the program was undertaken with the expectation that the chromosomes would be the biological sites most susceptible to radiation damage and that, therefore, C^{14} -labeled compounds that localized in the nucleic acids would represent perhaps the extreme of radiation hazard possible with carbon-14. These studies did in fact show that, following the intraperitoneal administration of tracer doses of a number of these precursors of nucleic acids, the resulting radiation of the chromosomes in some parts of the animal body did remain above the permissible level for several days.

Along with these studies, there was carried out an extensive study of the renewal of nucleic acid purines of a number of tissues of the animal body. These results are given in our 1956 report and in the 1957 report, which accompanies this proposal. Briefly summarized, in these studies sodium formate- C^{14} was administered to tumor-bearing animals to label the nucleic acids, and the loss of carbon-14 from the nucleic acids of a number of tissues was then studied over a period of time. Whereas rapidly dividing normal cells such as intestine and spleen lost carbon-14 from the nucleic acids either as a result of metabolic turnover or a physical loss of cells, no carbon-14 was lost over the period of the experiment from the nucleic acids of the tumor. These results suggested strongly that the tissues

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that are rapidly increasing in mass conserve their nucleic acids, and hence also conserve any carbon-14 that has been incorporated into the nucleic acids. At about the same time that these experiments were carried out a large number of other papers appeared that tend to substantiate the conservation of nucleic acids in a number of biological systems. Thus, among many references on the subject, one might cite evidence for the conservation of DNA in regenerating liver of rats^{1, 2} and in the liver of young rats³ and for the conservation of both DNA and RNA in bacteria^{4, 5}, mammalian cells in tissue culture^{6, 7}, and Ehrlich ascites tumor cells in vivo⁸. These results have led many to characterize the DNA of rapidly proliferating systems as "metabolically stable."

We have interpreted our own results (cf. the accompanying progress report) to indicate a dynamic equilibrium between nucleic acids and soluble nucleotides rather than true metabolic stability; however, regardless of interpretation, both our results and those of the other groups cited above clearly indicate retention of activity in nucleic acids.

Pertinent also is the work of Furst et al.^{9, 10} and Fresco, Bendich, and Russell¹¹ who gave partially-hepatectomized animals labeled precursors and studied, in the post-regeneration period, the retention of the

¹ O. Nygaard and H. P. Rusch, *Cancer Research* 15, 240 (1955).

² A. D. Barton, *Federation Proc.* 13, 422 (1954).

³ K. Kihara, M. Amano, and A. Sibatani, *Biochim. Biophys. Acta.* 21, 489 (1956).

⁴ A. D. Hershey, *J. Gen. Physiology* 38, 145 (1956).

⁵ L. Siminovitch and A. F. Graham, *Can. J. Microbiol.* 2, 585 (1956).

⁶ G. M. Healy, L. Siminovitch, R. C. Parker, and A. F. Graham, *Biochim. Biophys. Acta.* 20, 425 (1956).

⁷ R. Y. Thomson, T. Paul, and J. N. Davidson, *Biochim. Biophys. Acta.* 22, 581 (1956).

⁸ L. Revesz, A. Forssberg, and G. Klein, *J. Natl. Cancer Inst.* 17, 37 (1956).

⁹ S. S. Furst, P. M. Roll, and G. B. Brown, *J. Biol. Chem.* 183, 251 (1950).

¹⁰ S. S. Furst and G. B. Brown, *J. Biol. Chem.* 191, 239 (1951).

¹¹ J. R. Fresco, A. Bendich, and P. J. Russell, Jr., *Federation Proc.* 14, 214 (1955).

isotope that was incorporated during regeneration. They showed that after regeneration essentially no DNA was lost from the liver over a long period. Thus, it would appear that DNA is conserved not only during periods of rapid growth but also in non-dividing tissues such as liver of adult animals.

The evidence that DNA is conserved during the phases of rapid growth of such a variety of biological systems led us to wonder if embryonic tissues, which show many metabolic characteristics similar to tumor cells, would also be similar to tumor cells in the conservation of nucleic acids. Such thinking led to the preliminary and inconclusive experiments with pregnant mice described in the accompanying progress report and is the basis of our plans for continuation of this work.

The importance of the concept of "metabolic stability" of nucleic acid to radiation hazards is obvious. If indeed the foetus is similar to other rapidly growing systems in the conservation of nucleic acids then, in some tissues, activity incorporated in the nucleic acids during embryonic growth may remain there for the life of the individual. Thus, certain cells of the body would be exposed over a long period of time to radiation concentrated in the most sensitive locus, i. e., the genes and chromosomes. If the carbon-14 were introduced in the early stages of foetal development, then one might expect the maximum of radiation damage; i. e., initial exposure to radiation at a stage known to be extremely sensitive to radiation followed by a prolonged exposure of certain tissues after birth and during the maturing of the individual.

4. Proposed Research

Research proposed for the next year is based upon the considerations of conservation of nucleic acids which were discussed above. First, it is proposed to continue and expand the study of conservation of nucleic acids in embryonic tissue, with the purpose of establishing definitely whether embryonic tissue is similar to other rapidly dividing systems and further of attempting to settle the question of "metabolic stability" of nucleic acids versus dynamic equilibrium with soluble nucleotides. This part of the proposed program should provide results of fundamental biological and biochemical significance.

Consideration of the relationship of conservation of nucleic acids to the problem of carbon-14 hazard, as mentioned in the preceding section, leads directly to the more practical aspect of the program in which we propose to attempt to assess, by various means, the biological effects of carbon-14 introduced at an early stage of foetal development.

Specific experiments which will be carried out on the program as it can be envisioned at this stage are the following:

(a) Conservation of Nucleic Acids in Embryonic Tissue. Large groups of pregnant mice will be given injections of a C¹⁴-labeled precursor of purines and at various times thereafter during foetal development the purines of the RNA and DNA and the acid-soluble fraction will be assayed for carbon-14. Since the early stages of foetal development are known to be most sensitive, administration of C¹⁴-labeled compounds will be begun at the earliest feasible period following conception. The amount of carbon-14 in the nucleic acid purines at the various times will be a measure of conservation of the nucleic acids and a comparison of activities of nucleic acid purines and acid-soluble purines will determine whether conservation is due to true metabolic stability or whether it is accompanied by a dynamic equilibrium between nucleic acids and soluble precursors. In most of these experiments sodium formate-C¹⁴ will be used because of its cheapness and because it labels both adenine and guanine of both RNA and DNA. Other C¹⁴-labeled precursors will be used as results may indicate.

(b) If embryonic tissue should show conservation of carbon-14 in the nucleic acids, and the results now available suggest that it will fall in the same category as other rapidly growing systems, then long-term studies will be carried out on retention of the isotope during the lifetime of the animal. Such studies should show how long radioactivity, incorporated into nucleic acids of the foetus, will remain above the permissible level in the various organs of the animal after birth.

(c) The above experiments are tracer studies to determine the retention of activity in the nucleic acids. If retention is demonstrated, some experiments will be undertaken with quite a different emphasis: the maximum feasible amount of carbon-14 will be administered to pregnant mice and the litter will be observed for biological effects that might be attributed to radiation from carbon-14. Animals will be examined immediately after birth for obvious defects and then observed over their life span. Life spans of treated animals will be compared with those of untreated controls, and at death animals will be autopsied to discover less obvious biological effects that might be attributed to radiation. If damage from carbon-14 can be demonstrated, then experiments might be begun to determine the minimum level of carbon-14 that could cause demonstrable damage under these conditions. Depending on how initial experiments turn out, many other experiments might be suggested. For example, mice of the AK strain, of which essentially all animals develop leukemia, might be studied to determine whether carbon-14 introduced into the foetus affects the incidence or course of leukemia which develops after birth.

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The difference between these studies and any carried out earlier on this program should be emphasized. It was shown earlier that carbon-14 was rapidly lost from mammalian tissues after labeling with $\text{NaHC}^{14}\text{O}_3$ ¹ and that administration of sodium formate- C^{14} to leukemic mice failed to alter the course of the disease². However, these were experiments with adult mice that would be expected to incorporate and retain much less activity than animals receiving carbon-14 in the foetal stage of development.

To our knowledge, although many studies have been carried out on radiation hazard resulting from ingestion and retention of Sr^{90} and other radioactive isotopes, there have been no reports published on attempts to assess the hazards from carbon-14 resulting from its incorporation into nucleic acids of the foetus.

5. Scientific Personnel:

Principal Investigator

Howard E. Skipper, up to 10% of time, Ph. D. (Biochemistry).
Assistant Director in charge of Biological Sciences.

Experience: Chemical Warfare Service, 1941-1946: toxicology and mechanisms of action of chemical warfare agents, particularly alkylating agents. Southern Research Institute, 1946- : screening of agents for anticancer and antiviral activity; mechanism of action of anticancer agents; searches for exploitable biochemical differences between normal and cancer cells; mechanisms of resistance to anticancer agents.

Publications: Some eighty publications on cancer chemotherapy and related fields and, in addition, the following publications (supported by the subject grant) on hazards involved in use of C^{14} :

Skipper, White, and Bryan. Studies on the Hazard Involved in Use of C^{14} . I. Retention of Carbon from Labeled Bicarbonate. *J. Biol. Chem.* 180, 1187 (1949).

Skipper, White, and Bryan. Body Retention of Carbon-14 from Labeled Sodium Bicarbonate. *Science* 110, 306 (1949).

¹ H. E. Skipper, L. White, and C. E. Bryan, *J. Biol. Chem.* 180, 1187 (1949).

² H. E. Skipper, M. J. Bell, and J. B. Chapman, *Cancer Research* 10, 362 (1950).

Skipper, Bell, and Chapman. Studies on the Hazard Involved in the Use of C^{14} . II. The Effect of a Single Dose of C^{14} -Labeled Sodium Bicarbonate on the Pattern of Deaths from Spontaneous Leukemia in Akm Mice. *Cancer Research* 10, 362 (1950).

Skipper, Nolan, and Simpson. Studies on the Hazard Involved in Use of C^{14} . III. Long Term Retention in Bone. *J. Biol. Chem.* 189, 159 (1951).

Skipper. The Hazard Involved in the Use of Carbon-14. *Nucleonics* 10, 40 (1952).

Skipper, Simpson, and Bell. Long-Term Radiation of Bone Following Administration of C^{14} -Bicarbonate. *Proc. Soc. Exp. Biol. and Med.* 92, 549 (1956).

Other Scientific Personnel

L. L. Bennett, Jr., 10% of time, Ph. D. (Organic Chemistry), Head, Biochemistry Division.

Experience: Instructor in chemistry, University of Georgia, 1943-1944. Southern Research Institute, 1948- ; synthesis of C^{14} -labeled compounds; searches for biochemical differences between normal and cancer cells; mechanism of drug action; chemotherapy.

Publications: No publications directly relating to radiation hazard. Ten publications on synthesis of anticancer agents and C^{14} -labeled compounds and on nucleic acid metabolism.

Linda Simpson, 25% of time, B.S. Biophysicist, Biochemistry Division.

Experience: Southern Research Institute, 1948- ; low-level C^{14} assay in gas phase; construction and standardization of gas phase proportional counter for carbon-14 and tritium.

Publications: Co-author of papers on hazards in the use of carbon-14 (see publications of Skipper above).

J. Richard Thomson (not hitherto assigned to this project), 10% of time, M.S. (Cytology). Biologist, Chemotherapy Division.

Experience: Assistant bacteriologist, U. S. Public Health Service, 1949-50. Instructor, University of Chattanooga, 1950-51. Oak Ridge National Laboratory, 1951-52: incidence of leukemia due to radiation; biological effects of radiation. Southern Research Institute, 1952- : chemotherapy of cancer and leukemia.

Publications: Co-author of ten publications on chemotherapy of cancer.

6. Other Personnel:

One assistant chemist, 50% of time.

7. Other Financial Assistance:

The salaries of scientific personnel of this institution are paid out of industrial, federal, and institutional research contracts. Charges to each project are made on the basis of time spent on the project.

We have no other contracts concerned with the hazard from carbon-14; however, we are carrying on other programs on nucleic acid metabolism, and these programs support partially the studies on nucleic acid renewal.

8. Materials, Equipment, and Facilities:

Completely equipped animal rooms, biochemical laboratories, and tracer laboratories. No additional major equipment is needed. One of the biggest items is C¹⁴-labeled compounds; the amount indicated is an estimate and may be adjusted on the basis of results as they accumulate.

9. Travel:

No travel allowance is requested.

10. Budget

Salaries:

Biochemist (part time)	\$	1,980.
Biochemist, 10%		1,110.
Biologist, 15%		1,085.
Biophysicist, 25%		1,500.
Assistant chemist, 50%		1,800.
Total Salaries		<u>7,475.</u>

Contribution to Employees' Retirement Fund 285.

Materials and Supplies 1,500.

Radioactive compound	\$1,000.
Expendable glassware and animals	<u>500.</u>
	\$1,500.

Overhead (8% of total) 740.

SRI Contribution 5,003.

11. Amount Requested \$10,000.

Southern Research Institute's usual research contracts bear an overhead of 100% of scientific salaries. You will note, under the budget category, that we have computed overhead differential at the rate of 76.83% of salaries and wages, including vacation and sick leave pay. This figure is based on rate experience in 1956 and what we assume it will be in 1957-1958. The difference between 8% of direct costs and the rate of 76.83% indicates the extent of Southern Research Institute's support of the work outlined in the enclosed proposal.

12. Statement of Current Expenditures

(a) As of May 31, 1957, the total expenditures of the amount budgeted for the period 10/1/56 to 9/30/57 were:

AEC Participation	\$ 6,455.58
SRI Participation	<u>3,574.50</u>
	\$10,030.08

(b) Estimate of costs for the remainder of the contract is:

AEC Participation	\$ 5,376.62
SRI Participation	<u>2,477.13</u>
	\$ 7,853.75

13. Residual Funds

It is anticipated that all funds in present contract will be expended.


Howard E. Skipper
Assistant Director, Biological Sciences
Senior Investigator

Birmingham, Alabama
June 28, 1957
(6:5)
dh mw

C O P Y

S. R. Sapirie, Manager
Oak Ridge Operations Office

August 17, 1956

C. W. Shilling, M.D., Deputy Director
Division of Biology and Medicine

FY 1957 FUND AUTHORIZATION FOR NEGOTIATION OF DBM OFF-SITE CONTRACTS
FUNDED ON AN INTERIM BASIS DURING FY 1956

SYMBOL: BMP:NFS

For purpose of record this memorandum and the attachment hereto may be identified as contract authorization number BM-57-57.

Reference is made to my memorandum of July 18, 1956, subject as above. The attachment to this memorandum provides a detailed list of the remainder of such contracts funded on an interim basis. Funds for these contracts were included in Advice of Allotment Transferred, issued to your office under Allotment Symbol 06-71-91 (24) on August 10, 1956.

It is requested that your office take such action as may be necessary to extend and supplement each of these contracts for an additional contract period in the amount indicated in the attachment to this memorandum. The attachment includes the FY 1956 contract authorization number for each of these contracts; the Division's intent with respect to the contract period, level of support, and funding will be found in items 5 and 10 of each FY 1956 contract authorization.

There are attached 29 copies of form "Notification of Contract Negotiation" to cover the contracts included in this listing and 3 additional copies of the form for use in reporting negotiation of the contracts listed in the attachment to memorandum CA Number BM 57-17 dated July 18, 1956.

C O P Y

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FY 1957 FUND AUTHORIZATION FOR NEGOTIATION OF DBM OFF-SITE CONTRACTS FUNDED ON AN INTERIM BASIS DURING
 FY 1956 - AUGUST ALLOTMENT.

Contract Authorization No. BM-57-57

OAK RIDGE OPERATIONS OFFICE

FY 1956 C/A No.	Contractor	Contract Number	Effective Date of New 12 Months Period	Budget Category	FY 1956 Funds
56-345	Texas, Univ. of	40-1-1323	10/1/56 - 9/30/57	6130	\$ 24,840
56-348	Texas, Univ. of	40-1-1649	10/1/56 - 9/30/57	6130	4,410
56-425	Texas, Univ. of	40-1-1750	10/1/56 - 9/30/57	6130	6,000
56-333	Texas, Univ. of	40-1-1751	10/1/56 - 9/30/57	6130	9,000
56-301	Virginia, Univ. of	40-1-2010	10/1/56 - 9/30/57	6140	6,300
56-317	Duke University	40-1-289	10/1/56 - 9/30/57	6320	13,000
56-299	Rice Institute	40-1-284	10/1/56 - 9/30/57	6330	14,500
56-396	Southern Res. Institute	40-1-1038	10/1/56 - 9/30/57	6330	10,851
56-411	Texas, Univ. of	40-1-1040	10/1/56 - 9/30/57	6330	10,203
56-385	Texas Agric. Exp. Station	40-1-1758	10/1/56 - 9/30/57	6330	8,700
				Sub-total	\$107,804
56-307	Vanderbilt University	40-1-1033	11/1/56 - 10/31/57	6120	23,000
56-302	Wake Forest College	40-1-1638	12/1/56 - 11/30/57	6120	18,514
56-304	Yerkes Laboratory	40-1-1553	12/1/56 - 11/30/57	6120	19,335
56-117A	Tennessee, Univ. of	40-1-1999	11/1/56 - 10/31/57	6120	5,000
56-409	Duke University	40-1-1647	11/1/56 - 10/31/57	6130	22,990
56-323	Florida Univ. Agric. Exp. Sta.	40-1-2004	11/1/56 - 10/31/57	6130	5,700
56-349	North Carolina State College	40-1-1314	11/1/56 - 10/31/57	6130	6,400
56-382	North Carolina State College	40-1-1747	11/1/56 - 10/31/57	6130	17,379
56-369	Maharry Medical College	40-1-269	11/1/56 - 10/31/57	6310	40,000
56-397	Oklahoma Medical Res. Found.	40-1-1433	11/1/56 - 10/31/57	6320	11,000
56-320	Tennessee, Univ. of	40-1-1642	11/1/56 - 10/31/57	6320	13,000
56-313	Tennessee, Univ. of	40-1-1643	11/1/56 - 10/31/57	6320	18,804
56-357	Tennessee, Univ. of	40-1-2003	11/1/56 - 10/31/57	6320	6,000
56-372	Virginia Univ. Sch. of Med.	40-1-263	12/1/56 - 11/30/57	6320	7,020
56-426	Christian Brothers College	40-1-2005	11/1/56 - 10/31/57	6330	7,400
56-423	Florida, Univ. of	40-1-1321	11/1/56 - 10/31/57	6330	16,740
56-388	North Carolina State College	40-1-264	11/1/56 - 10/31/57	6330	7,800
56-384	North Carolina State College	40-1-1324	11/1/56 - 10/31/57	6330	6,834
56-351	Vanderbilt University	40-1-1322	11/1/56 - 10/31/57	6560	7,780
				Sub-total	260,696
				Total	\$368,500

Office Memorandum • UNITED STATES GOVERNMENT

TO : J. W. Guld, Jr., Assistant General Counsel DATE: June 13, 1956

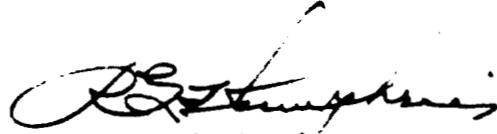
FROM : R. G. Humphries, Acting Director, Contract Division

SUBJECT: RENEWAL OF CONTRACT NO. AT-(40-1)-1038 - SOUTHERN RESEARCH INSTITUTE

SYMBOL: ACT:ARB

Enclosed is an approved proposal for renewal of subject contract for the period July 1, 1956 through September 30, 1957. This action is covered by Contract Authorization No. BM-56-396, dated June 8, 1956, in the amount of \$2,758.00.

Please prepare an appropriate modification to extend the term of this contract to September 30, 1957, with new funds in the amount of \$2,758.00, to be paid on a lump sum basis for the period July 1 through September 30, 1956; and with a further provision for additional funds in the amount of \$1,351.00 to be paid after written notification from the contract administrator. Provision for compliance with AEC Manual Chapter 7510 should also be included in this modification.


R. G. Humphries

Enclosures:

1. Request for Contract Action
2. Budget
3. Resume
4. Contract Authorization BM-56-396
5. Renewal Proposal

CC: C. S. Shoup
L. D. MacKay
J. Nicholson, w/cys Encls. 1 and 2

1. Mr. J. R. Moore Chairman
Contract Board. From: Res. & Dev. Div.

It is requested that the Contract Board take the necessary action to process the following described contract action in accordance with the provision of Bulletin OR-OM-14:

2. Nature of Action Requested

Selection of New Contractor and Negotiation of Contract.

Modification of Contract

No. AT-(40-1)-1038

Contractor: Southern Research Institute
Birmingham, Alabama

Review and approval of Contract, Sub-contract or Purchase Order.

Other (Explain) _____

Number: _____

Name: _____

*OK
4/13*

3. Nature of Services to be Covered by Contract

Construction

Architect-Engineer

Other

(Explain) Research

4. Funding

Amount to be Obligated by this Contract Action \$ 2,758.00

Source of Funds

Approved ORO Financial Plan, _____ Quarter, Fiscal Year 19__

Project No. _____ or, Activity No. 6330

Funds to be Obligated: Allotment No. 06619(64) F.Y. 1956 Funds

Procurement Directive No. BM 56-396 Dated 6-8-56

Issuing Office Div. Biology & Medicine

Concurrence in Funding Statement: (signed) _____

Joseph L. Petty
Chief, Budget Branch 6/13/56

5. Project or Activity to be Covered by Contract Action:

Location of Work: _____ Construction Directive No. _____

Estimated Cost of Work to be Covered by this Contract Action \$ _____

Schedule: Date Work to Start _____ Estimated Completion Date _____

Description of Project or Activity: _____

If more space is required use separate sheets and attach hereto:

6. Contract Board Docket
No. _____
To be assigned by
Board's Secretary) _____

7. Request Submitted By: (signed) _____
Date: _____ Title: _____

JUN 19 1956

8. Complete Description of Services to be Furnished by Contractor:
Washington designated research contract.

Title: "Body Retention of Carbon-14".

(If more space is required use separate sheets and attach hereto:)

9. Description of other changes to be covered by Modification:

Modify contract to provide for extension of period to September 30, 1956, with new funds in the amount of \$2,758. Include provisions for compliance with AEC Manual Chapter 7510. Also include provisions for extension of period to September 30, 1957, with additional funds in the amount of \$10,851, subject to further written notification from the contract administrator. Provide for lump-sum payment of funds for first period.

10. Negotiated Contracts. (Show why it appears desirable to negotiate new contract or to negotiate modification to existing contract)

Memorandum from C. W. Shilling to S. R. Sapirie, dated June 8, 1956.

(If more space is required use separate sheets and attach hereto:)

11. Contracts, Subcontracts, or Purchase Orders Submitted for Review and Approval: (Furnish brief description of action in this space and attach pertinent documents)

None

12. Disputes:

Attach a statement summarizing the dispute together with pertinent documents and Background Material.

None

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BUDGET FOR CONTRACT NO. AT-(40-1)-1038
FOR PERIOD 7-1-56 - 9-30-56

<u>Salaries and Wages:</u>		\$2,253.80
Dr. Howard E. Skipper	\$ 825.00	
Research Associate and Assistants	1,363.80	
Retirement Fund	65.00	
<u>Materials and Supplies:</u>		300.00
<u>Indirect Costs:</u>		1,713.87
		<hr/>
		\$4,267.67

The Commission's contribution to the above budget will be \$2,758.

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BUDGET FOR CONTRACT NO. AT-(40-1)-1038
FOR PERIOD 10-1-56 - 9-30-57

<u>Salaries and Wages:</u>		\$ 8,998.20
Dr. H. E. Skipper	\$3,300.00	
Research Associate and Assistants	5,455.20	
Retirement Fund	243.00	
(2) <u>Materials and Supplies:</u>		1,050.00
(3) <u>Indirect Costs:</u>		6,855.47
		<hr/>
	TOTAL:	\$16,903.67

The Commission's contribution to the above budget will be \$10,851.

SOUTHERN RESEARCH INSTITUTE
BIRMINGHAM, ALABAMA

DR. HOWARD E. SKIFFER, Project Leader, CONTRACT NO. AT-(40-1)-1038

BODY RETENTION OF CARBON-14

Resume'

During the seventh period of performance, the Contractor shall
~~For the next contract period it is proposed to~~ continue work along the lines already underway; i.e., a study of the tissue radiation resulting from administration of additional C^{14} -labeled precursors of nucleic acids. C^{14} -labeled compounds available for this work and not hitherto studied are: adenylic acid (mixture of 2' - and 3' -isomers), adenosine-5'-phosphate, inosine-5'-phosphate, xanthylic acid (mixture of 2'-and3'-isomers), adenosine, guanosine, inosine, and xanthosine. These compounds will be administered to mice, rats, and hamsters bearing tumors, and the radiation in each of several tissues resulting from the administration of these compounds will be calculated on the basis of the incorporation at six hours and the known turnover of the nucleic acid purines.

C. S. Shoup

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UNITED STATES ATOMIC ENERGY COMMISSION
 WASHINGTON, D. C.

Contract Authorization No. BM-56-396

JUN 8 1956

TO : S. R. Sapirie, Manager
 Oak Ridge Operations Office

FROM : C. W. Shilling, M. D., Deputy Director
 Division of Biology and Medicine, Washington, D.C.

SUBJECT : FUND AUTHORIZATION AND TRANSMITTAL OF RESEARCH PROPOSAL FOR
 CONTRACT NEGOTIATION

REFERENCE : AEC 102/16 APPROVED OCTOBER 7, 1953, AS IMPLEMENTED BY MEMORANDUM
 TO MANAGERS, OPERATIONS OFFICES, DATED OCTOBER 23, 1953, JOINTLY
 SIGNED BY THE DIRECTORS OF THE DIVISIONS OF RESEARCH AND
 BIOLOGY AND MEDICINE.

SYMBOL : EMB:JRT

The research proposal described below has been approved by the
 Division of Biology and Medicine, funds are available, and you
 are authorized and requested to negotiate a contract in
 accordance with the following terms and conditions:

1. Institution: Southern Research Institute
2. Investigator (s): Dr. Howard E. Skipper
3. Title: Body Retention of Carbon-14
4. () New Contract, (x) Renewal of Contract No. AT(40-1)1038
5. Duration:

7-1-56 thru 9-30-57	\$13,509
7-1-56 thru 9-30-56	2,758
6. AEC Technical Representative:

10-1-56 thru 9-30-57	\$10,851
----------------------	----------

 Dr. John R. Totter *JRT*
7. Funds are authorized for the obligation of this contract
 as follows:

<u>Allotment No.</u>	<u>Budget Category</u>	<u>Previous</u>	<u>Amount</u>	
			<u>This Action</u>	<u>Total</u>
06-61-91 (24)	6330		\$2,758	\$2,758
Please see item #10				
_____	_____	_____	_____	_____

JUN 1 1956 7-4962

8. It is suggested that in the best interests of the government the following type contract be negotiated: lump-sum
9. It is requested that the title to any capital equipment procured under this contract shall be vested with:

(x) the contractor; () the government.

10. Other comments: The balance of approved contract funds in the amount of \$10,851 for the contract period 10-1-56 thru 9-30-57 will be allotted promptly upon receipt of FY 1957 appropriations. It is recommended that the contract extension be for the full period approved in item 5, with provision for funding as indicated above.

If radioisotopes are to be used in this research, it is requested that the savings available to the contractor under the Radioisotope Research Support Program (Ref. AEC Manual Chapter 7510) be considered in the negotiation of the amount to be funded under this contract.

11. Security Requirements:

In accordance with the provisions of Chapter 3403 of the AEC Manual and the requirements of the Declassification Guide, it has been determined that the following security precautions should be taken in connection with the proposed research contract:

Since there is essentially no chance for the development of restricted data, this project has been placed in Category I as defined in Chapter 3403 of the AEC Manual.

12. Reports: (x) Reports are to be required as provided for by "Revised Guide for the Submission of Research Proposals" dated February 8, 1954.

() Special reports instructions are as follows:

Enclosures: (x) "A" - Proposal, dated March 30, 1956
(x) "B" - Notification letter, dated JUN 8 1956
() "C" - Other correspondence, _____ letters

Distribution:

Addressee: Original (w encl.) Division File: Yellow copy (w encl.)
1st copy (w encl.) Pink copy (w/o encl.)
2nd copy (w encl.)

Branch File: White copy (w encl.)

Program Analysis Branch:
White copy (w/o encl.)

REQUEST FOR RENEWAL OF CONTRACT

NO. AT-(40-1)-1038

ON

BODY RETENTION OF CARBON 14

FOR

ATOMIC ENERGY COMMISSION

DIVISION OF BIOLOGY AND MEDICINE

SOUTHERN RESEARCH INSTITUTE

Birmingham, Alabama

March 30, 1956

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REQUEST FOR RENEWAL OF CONTRACT NO. AT-(40-1)-1038

ON

BODY RETENTION OF CARBON-14

1. Title of the Project: Body Retention of Carbon-14
2. Institution and Department: Southern Research Institute, Biochemistry Division.
3. Scientific Background.

In view of the wide use of carbon-14 in laboratory studies and the increasing use of carbon-14 in human tracer studies, it has been considered worthwhile to continue the study of the retention of this isotope in various tissues and organs (and areas of isotope concentration in tissues and cells) following administration of various carbon-14-labeled organic compounds.

Previously a rather complete study was carried out on the long-term retention and tissue radiation following administration of $C^{14}O_3$ to animals.

For the past two years efforts have been made to learn more about the localization and turnover of C^{14} -labeled compounds which are known to be precursors of genetic components of various animal cells and human tumors (growing in cortisonized hamsters or rats).

It should be made clear that these studies have more than this one objective. We are vitally interested in nucleic acid metabolism and turnover in a variety of normal tissues of animals and in potentially exploitable biochemical differences in nucleotide and nucleic acid metabolism between normal and neoplastic tissues. Such information could supply a key to the planned synthesis of more effective anticancer agents. Also it should be made clear that our tracer program, which is planned so that tissue turnover and tissue radiation calculations and fundamental biochemical information can be obtained, derives support from the subject grant (AT-(40-1)-1038) and grants from the C. F. Kettering and the Alfred P. Sloan Foundations. We consider it fortunate that in addition to data coming from the smaller effort from project AT-(40-1)-1038, the rather large amount of data from other tracer programs are available for retention and tissue radiation evaluations.

During the past year under the contract, data have been obtained on tissue radiation following administration of a number of C^{14} -labeled compounds (glycine, formate, 4-amino-5-imidazolecarboxamide, hypoxanthine, adenine, guanine, 2,6-diaminopurine, and guanylic acid) to tumor-bearing animals. These data are presented in detail in the accompanying progress report. Two papers have been completed during the past year which represent results made possible by these tracer studies.

1. Bennett, Skipper, Stock, and Rhoads, Searches for Exploitable Biochemical Differences between Normal and Cancer Cells. I. Nucleic Acid Purine Metabolism in Animal Neoplasms. *Cancer Research* 15, 485-91 (1955).

2. Bennett, Skipper, Toolan, and Rhoads, Searches for Exploitable Biochemical Differences between Normal and Cancer Cells. II. Nucleic Acid Purine Metabolism in Human Tumors. *Cancer Research*, in press.

4. Proposed Research.

For the next contract period it is proposed to continue work along the lines already underway; i. e., a study of the tissue radiation resulting from administration of additional C^{14} -labeled precursors of nucleic acids. C^{14} -labeled compounds available for this work and not hitherto studied are: adenylic acid (mixture of 2'- and 3'-isomers), adenosine-5'-phosphate, inosine-5'-phosphate, xanthylic acid (mixture of 2'- and 3'-isomers), adenosine, guanosine, inosine, and xanthosine. These compounds will be administered to mice, rats, and hamsters bearing tumors, and the radiation in each of several tissues resulting from the administration of these compounds will be calculated on the basis of the incorporation at six hours and the known turnover of the nucleic acid purines.

It appears to us that these studies are of possible value to the AEC if:

- (a) Turnover and tissue radiation data in animals following injection of a variety of organic C^{14} -labeled compounds are of interest.
- (b) Fundamental data on nucleic acid metabolism in animal tissue are pertinent to the program of the AEC.
- (c) Fundamental studies on the nucleotide and polynucleotide metabolism of normal and neoplastic tissues are of interest.

It is difficult for us to judge the degree of interest of the Commission in these problems and thus the reasonableness of our request for continued support. To our knowledge this is one of the few programs now in existence which is seeking knowledge which might be used as a guide for the setting of allowable levels for human experimentation with various C¹⁴-labeled organic compounds of considerable interest in human metabolism.

5. Scientific personnel:

Howard E. Skipper, Ph.D. Head, Biochemistry Division	Part time
L. L. Bennett, Jr., Ph.D., Biochemist	10%
Patricia Morgan, B.S., Chemist	75%
Sally Johnson, B.S., Biologist	25%
Linda Simpson, B.S., Biophysicist	12%

6. No significant amount of time will be charged by other personnel.

7. Other Financial Assistance:

The salaries of scientific personnel of this institution are paid out of industrial, federal, and institutional research contracts. Charges to each project are made from daily time sheets showing distribution of effort. We have no other contracts concerned with the hazard involved in the use of carbon-14; however, we are carrying on other programs having to do with nucleic acid metabolism.

8. Materials, Equipment and Facilities:

Completely equipped animal rooms, biochemical laboratories, and tracer laboratories. **No additional major equipment is required.** Radioactive compounds will be purchased as required.

9. Travel.

No travel allowance is requested.

10. Budget:

A. From 7/1/56 to 9/30/56

Salaries:

Biochemist (part time)	\$	825.00
Biochemist (part time)		234.00
Chemist (part time)		720.00
Biophysicist (part time)		154.80
Biologist (part time)		<u>255.00</u>

Total Salaries \$2,188.80

Contribution to Employees' Retirement Fund 65.00

Materials and Supplies:

Radioactive Compounds	\$150	
Glassware and animals	<u>150</u>	<u>300.00</u>

Subtotal \$2,553.80

Overhead (8% of above) 204.30

S. R. I. Contribution 1,509.57
\$4,267.67

B. From 10/1/56 to 9/30/57

Salaries:

Biochemist (part time)	\$3,300.00
Biochemist (part time)	936.00
Chemist (part time)	2,880.00
Biophysicist (part time)	619.20
Biologist (part time)	<u>1,020.00</u>

Total Salaries \$8,755.20

Contribution to employees' Retirement Fund 243.00

Materials and Supplies:

Radioactive Compounds	\$600	
Glassware and Animals	<u>450</u>	<u>1,050.00</u>

Subtotal 10,048.00

Subtotal (Brought Forward)	\$10,048.00
Overhead (8% of above)	803.84
S. R. I. Contribution	<u>6,051.63</u>
	\$16,903.47

11. Amount Requested.

The Southern Research Institute's usual research contracts bear an overhead of 100% of scientific salaries. You will note, under the budget category, that we have computed overhead differential at the rate of 83% of salaries and wages, exclusive of vacation and sick leave pay. This figure is based on rate experience in 1955 and what we assume it will be in 1956-1957. The difference between 8% of direct costs and the rate of 83% indicates the extent of Southern Research Institute's support of the work outlined in the enclosed proposal.

12. Statement of Current Expenditures.

(a) As of February 29, 1956, total expenditures were:

AEC participation	\$7,474.00	
SRI participation	<u>2,742.05</u>	
		\$10,216.05

(b) Estimate of costs for the remainder of the contract is:

AEC participation	\$2,526.00	
SRI participation	<u>2,414.95</u>	
		<u>4,940.95</u>
		\$15,157.00

(c) We do not expect to have any balance of funds from the current contract period available for financing project during proposed period of performance.

Howard E. Skipper (by L. L. R.)
Howard E. Skipper, Assistant Director
Senior Investigator

Birmingham, Alabama
March 30, 1956
10 (kb)

Office Memorandum • UNITED STATES GOVERNMENT

TO : R. G. Humphries, Contract Coordinator

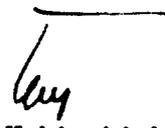
FROM : T. M. Yakinchick, Special Assistant to the Director

SUBJECT: SOUTHERN RESEARCH INSTITUTE OVERHEAD

SYMBOL: F:TM

DATE: June 23, 1950

An examination of the operating statement submitted by Southern Research Institute for the Calendar Year 1949 indicates an overhead rate of 97.06 percent based on direct labor. Salaries of technical personnel (direct labor) amounted to \$193,749.38, and general and administrative expenses, which excludes materials, supplies, and services not charged directly to sponsored projects, amounts to \$188,090.98.


T. M. Yakinchick

In Reply Refer to:
CO:R&E

Oak Ridge, Tennessee
June 14, 1950

Dr. Edward E. Snijder
Assistant Director
Southern Research Institute
Birmingham 1, Alabama

Subject: CONTRACT NO. 49-(10-1)-2020

Dear Dr. Snijder:

Your research project which was submitted to the Commission's Division of Biology and Medicine, Washington, D. C., has been approved by that office and has been forwarded to this office for preparation of an appropriate contract covering the Commission's support of your project.

Enclosed, in three copies, is a contract numbered as shown in the subject line above, which incorporates in Appendix "B" a description of your project and the budget for the first period which you are to follow as a general guide.

Your attention is called to the item in the budget covering the allowance for overhead. If you feel the amount allocated to overhead can be more effectively used on this project in purchasing additional equipment, supplies or time of personnel this office has no objection to such a change provided you can reach agreement on this change in the budget with your Business Manager. The use of funds supplied by the Commission to cover your direct costs is favored over the use of such funds to defray a part of your established overhead costs. If you reach such an agreement with your business representative it is requested that you make the necessary changes in the budget in ink. The changes thus made should be initialed by you, as Project Leader, and by the party signing the contract for the Institute.

It is requested that you sign each copy of the contract in the space provided for the Project Leader on the signature page of the contract and have the contract signed by the proper official of the Institute. All copies should then be returned to this office. After the Contracting Officer has signed the contract for the Commission a fully executed copy of the contract, together with one conformed copy, will be returned for the use of the Institute.

1111347

June 14, 1950

It will be noted that the contract provides for payment in Article III of a lump sum in consideration of your performance of the research activities described in Appendix "A". The first payment, representing one-half the amount of the agreed compensation, will be paid to you upon your submission of a properly certified voucher on or before the first date established in Article II of the contract. The remaining 50% of the agreed compensation will be paid to you within six months from the date of the first payment.

Performance of a cost audit of your expenditures has been eliminated through this lump sum payment for your research services. It is believed that this will save you considerable time and trouble in detailing your expenditures on cost reimbursement vouchers.

In order to assist you in preparing an appropriate voucher there is enclosed an instruction sheet containing important instructions. Instructions with numbers appearing on a specimen copy of the voucher form should be submitted to the Oak Ridge Operations office in one original (white) and four copies (yellow) addressed as shown in Article II of the contract. It is assumed that you will give your business office the benefit of these instructions.

Your attention is called to the reporting requirements outlined in Appendix "C" to the contract, especially to Item No. 2 requiring the immediate submission of a 200 word summary statement describing the purpose and scope of your project.

For your information and guidance in purchasing isotopes through the Commission, in accordance with the provisions of Article VII, there is enclosed a copy of the latest radioisotope catalog, together with a set of application forms, which you will use in making purchases of isotopes.

In connection with the provisions of Section J of Appendix "B", which require the person directing the research work under this contract to be cleared by the Commission for access to restricted data, there is enclosed a set of Personnel Security Questionnaire forms, Fingerprint Card, Security Acknowledgment and Instructions therefor, to be filled out by you as Project leader and returned to this office.

Your particular attention is invited to Appendix "B", Section II - Personnel

It is believed that the remaining portions of the contract are self-explanatory, however, if you have any questions concerning the application or interpretation of any of the contract provisions this

Southern Research Institute

- 3 -

June 14, 1950

office will be glad to furnish you with additional information
pertaining thereto.

Very truly yours,

G. Ruden Bush
Assistant to the Manager
Oak Ridge Operations

Enclosures
1. Lactose Catalog & Applic. form w/instr. sheet
2. POC Form, Payment Card, Sec. Acknowledgment
3. Lactose sheet (2.000)

CC: Paul E. Pearson (Washington)
Harry Steebie (Oak Ridge)

CC: A. H. Corley
S. Sobel
G. Nicholson

Enclosure, in



1111349

Office Memorandum • UNITED STATES GOVERNMENT

TO : J. W. Culd, Jr., Assistant General Counsel DATE: May 25, 1955

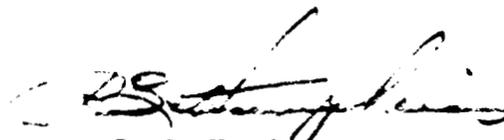
FROM : R. G. Humphries, Assistant Director, Contract Division

SUBJECT: RENEWAL OF CONTRACT NO. AT-(40-1)-1038 - SOUTHERN RESEARCH INSTITUTE

SYMBOL: ADA:ARB

Enclosed is an approved proposal for renewal of subject contract for a period of one year beginning July 1, 1955. This action is covered by Contract Authorization No. BM-55-320, dated April 29, 1955, in the amount of \$10,000.00.

Please prepare an appropriate modification to renew this contract for a period of one year, beginning July 1, 1955, with additional funds in the amount of \$10,000.00.



R. G. Humphries

Enclosures:

1. Request for Contract Action
2. Budget for Contract AT-(40-1)-1038
3. Resume
4. Cy Ltr fm Skipper to Roth dtd 5/17/55
5. Contract Authorization BM-55-320
6. Cy Ltr fm Skipper to Shoup dtd 4/11/55
7. Renewal Proposal

CC: C. S. Shoup
 L. D. MacKay
 J. Nicholson, w/Encls. 1 and 2

Brown:arb

1. Chairman
TO: J. R. Moore Contract Board. From: Research & Medicine Div.

It is requested that the Contract Board take the necessary action to process the following described contract action in accordance with the provisions of Bulletin OR-O&M-19:

2. Nature of Action Requested

Selection of New Contractor and Negotiation of Contract.

Modification of Contract No. AT-(40-1)-1038

Contractor: Southern Research Institute Birmingham 5, Ala.

Review and approval of Contract, Sub-contract or Purchase Order.

Other (Explain)

Number: _____
Name: _____

3. Nature of Services to be Covered by Contract

Construction

Architect-Engineer

Other

(Explain) Resources

4. Funding

Amount to be Obligated by this Contract Action \$ 10,000.

Source of Funds

Approved ORO Financial Plan, _____ Quarter, Fiscal Year 19__

Project No. _____ or, Activity No. 6460

Funds to be Obligated: Allotment No. 66-57-9(24) F.Y. 1955 Funds)

Procurement Directive No. BM-55-320 Dated 4-29-55

Issuing Office Div. of Biology & Medicine

Concurrence in Funding Statement: (signed)

Joseph T. Potter
Chief, Budget Branch 5/24/55

5. Project or Activity to be Covered by Contract Action:

Location of Work: _____ Construction Directive No. _____

Estimated Cost of Work to be Covered by this Contract Action \$ _____

Schedule: Date Work to Start _____ Estimated Completion Date _____

Description of Project or Activity:

(If more space is required use separate sheets and attach heretofore)

<p>6. <u>Contract Board Docket</u> No. _____ (To be assigned by Board Secretary)</p>	<p>7. <u>Request Submitted By:</u> <i>(Signature)</i> Date: <i>5-23-55</i> Title: <i>Herman M. Roth</i> HERMAN M. ROTH DIRECTOR RESEARCH AND MEDICINE DIVISION</p>
<p>8. <u>Complete Description of Services to be Furnished by Contractor:</u> Washington designated research contract. Title: "Body Retention of Carbon-14." (If more space is required use separate sheets and attach hereto)</p>	
<p>9. <u>Description of other changes to be covered by Modifications:</u> Renew contract for a period of one year beginning July 1, 1955, with Commission funds in the amount of \$10,000. (If more space is required use separate sheets and attach hereto)</p>	
<p>10. <u>Negotiated Contracts.</u> (Show why it appears desirable to negotiate new contract or to negotiate modification to existing contract) Memo J. C. Bugher to S. R. Sapirie dated April 29, 1955. (If more space is required use separate sheets and attach hereto)</p>	
<p>11. <u>Contracts, Subcontracts, or Purchase Orders Submitted for Review and Approval:</u> (Furnish brief descrip- tion of action in this space and attach pertinent documents) None</p>	
<p>12. <u>Disputes:</u> Attach a statement summarizing the dispute together with pertinent documents and Background Material. None</p>	

OK
H
5/25

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BUDGET FOR CONTRACT NO. AT-(40-1)-1038

FOR PERIOD 7-1-55 - 6-30-56

(1) <u>Salaries & Wages;</u>		\$ 7,934.00
Dr. H. E. Skipper (15% of time)	\$2,000.00	
Research Assistants	5,934.00	
(2) <u>Retirement Fund:</u>		275.00
(3) <u>Materials & Supplies;</u>		1,050.00
(4) <u>Overhead:</u>		5,898.00
	TOTAL	<u>\$15,157.00</u>

The Commission's contribution to the above budget will be \$10,000.

1111353

SOUTHERN RESEARCH INSTITUTE
BIRMINGHAM, ALABAMA.

Dr. Howard Skipper,
Project Leader

CONTRACT NO. AT-(40-1)-1038.

BODY RETENTION OF CARBON-14

Resume'

by The Contractor

Work will continue on incorporation of C-14 from various labelled compounds into genetic chemicals of normal animal tissues and human tissues growing in animals, with view of the work being of value in setting limits on certain classes of C-14-labeled compounds in human tracer studies. It is hoped that information can be gained of value in identifying the nucleic acid metabolism characteristics of human tumor tissue. Further studies will be planned on human-tumor bearing animals with the following C-14 compounds:

C-14 Thymine	C-14 Adenylic Acid
C-14 Uracil	C-14 Thymidine
C-14 Orotic Acid	C-14 Serine
C-14 Aspartic Acid	C-14-4-Amino-5-imidazolecarboxamide
C-14 Guanylic Acid	C-14-Inosinic Acid.

C. S. Shoup

Southern Research Institute

BIRMINGHAM 5, ALABAMA

May 17, 1956

Dr. Herman A. Call
Director, Research and Development Division
U. S. Atomic Energy Commission
Pet. Bldg., Tennessee

Dear Dr. Call:

Thank you for your letter of May 4 regarding Contract No. At-(49-1)-1038. As per your suggestion we have revised the budget, and reduced the total cost to \$15,157 which represents a contribution of \$10,000 by the AEC and \$5,157 by Southern Research Institute. Enclosed herewith is a copy of the revised budget.

If there are any further questions concerning the negotiation of this contract, please do not hesitate to call on us.

Yours sincerely,

Edward E. Snider
Assistant Director

ENC. 1
L21

E-3176

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**Summary of Direct and Indirect Costs
For One Year**

1. Salaries

Engineers, Scientists, Technicians
and Clerical (See Item 1)

2. Contributions to Retirement Allowances

Pay

Minimum Company

Maximum and Minimum

3. Overhead (5% of above)

S. S. I. overhead contribution to contract.
(Difference between 75.0% of direct salaries
(Less vacation and sick leave), and A. E. C.
overhead of 5%)

8,157

TOTAL

8,157

Recommended rate by Army Audit Agency
based on 1954 operation.

UNITED STATES ATOMIC ENERGY COMMISSION
WASHINGTON, D. C.

Contract Authorization No. EM-55-320

TO : S. R. Sapirie, Manager
Oak Ridge Operations Office

APR 29 1955

FROM : Dr. John C. Bugher, Director, Division of
Biology and Medicine, Washington, D.C.

SUBJECT : FUND AUTHORIZATION AND TRANSMITTAL OF RESEARCH PROPOSAL FOR
CONTRACT NEGOTIATION

REFERENCE : AEC 102/16 APPROVED OCTOBER 7, 1953, AS IMPLEMENTED BY MEMORANDUM
TO MANAGERS, OPERATIONS OFFICES, DATED OCTOBER 23, 1953, JOINTLY
SIGNED BY THE DIRECTORS OF THE DIVISIONS OF RESEARCH AND
BIOLOGY AND MEDICINE.

SYMBOL : BMB:PBP

The research proposal described below has been approved by the
Division of Biology and Medicine, funds are available, and you
are authorized and requested to negotiate a contract in
accordance with the following terms and conditions:

1. Institution: SOUTHERN RESEARCH INSTITUTE
2. Investigator (s): DR. HOWARD E. SKIPPER
3. Title: BODY RETENTION OF CARBON-14.
4. () New Contract, (x) Renewal of Contract No. AT(40-1)1038
5. Duration: July 1, 1955 to June 30, 1956
6. AEC Technical Representative: Dr. Paul B. Pearson
7. Funds are authorized for the obligation of this contract
as follows:

<u>Allotment No.</u>	<u>Budget Category</u>	<u>Previous</u>	<u>Amount This Action</u>	<u>Total</u>
06-51-91 (24)	6400		\$10,000	\$10,000
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____

MAY 2 1955
E-2627

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- 8. It is suggested that in the best interests of the government the following type contract be negotiated: Lump-sum
- 9. It is requested that the title to any capital equipment procured under this contract shall be vested with:

(x) the contractor; () the government.

10. Other comments:

The committee recommended approval of this project at the \$10,000 level, which is somewhat lower than requested.

11. Security Requirements:

In accordance with the provisions of Chapter 3403 of the AEC Manual and the requirements of the Declassification Guide, it has been determined that the following security precautions should be taken in connection with the proposed research contract:

Since there is essentially no chance for the development of restricted data, this project has been placed in Category I as defined in Chapter 3403 of the AEC Manual.

- 12. Reports: (x) Reports are to be required as provided for by "Revised Guide for the Submission of Research Proposals" dated February 8, 1954.

() Special reports instructions are as follows:

- Enclosures: (x) "A" - Proposal, dated Rec'd from Oak Ridge
 (x) "B" - Notification letter, dated APR 29 1955
 () "C" - Other correspondence, _____ letters

Distribution:

- | | |
|-------------------------------|--------------------------------------|
| Addressee: Original (w encl.) | Division File: Yellow copy (w encl.) |
| 1st copy (w encl.) | Pink copy (w/o encl.) |
| 2nd copy (w encl.) | Branch File: White copy (w encl.) |
| Program Analysis Branch: | |
| White copy (w/o encl.) | |

Southern Research Institute



BIRMINGHAM 5, ALABAMA

April 14, 1955

Dr. C. S. Shoup
Chief, Biology Branch
Research and Medicine Division
United States Atomic Energy Commission
Oak Ridge, Tennessee

Dear Dr. Shoup:

In answer to your letter of April 7, reference Contract No. AF-(40-1)-1038, the following additional information is submitted which we hope will clarify the situation.

- (1) As of March 31, 1955 total expenditures were
A.E.C. participation \$7,090.88
S.R.I. participation 3,619.71
\$10,670.59

- (2) Estimate of costs for the remainder of contract is
A.E.C. participation \$4,949.12
S.R.I. participation 2,770.18
\$7,720.30
\$18,390.89

- (3) We do not expect to have any appreciable balance of funds available for financing project during period of performance.

Please disregard item 12 in our proposal for renewal of the subject contract (date March 31, 1955). I was confused in my understanding of the present budget standing.

With apologies for this confusion,

Yours sincerely,

Howard E. Skipper
Assistant Director

hes pj

APR 18 1955

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E-2271

Southern Research Institute



BIENINGHAM 1 ALABAMA

April 14, 1955

Dr. C. S. Shoup
Chief, Biology Branch
Research and Medicine Division
United States Atomic Energy Commission
Oak Ridge, Tennessee

Dear Dr. Shoup:

In answer to your letter of April 7, reference Contract No. AT-(40-1)-1032, the following additional information is submitted which we hope will clarify the situation.

- (1) As of March 31, 1955 total expenditures were
A.E.C. participation 87,880.88
S.R.I. participation 16,886.77
\$10,670.55

- (2) Estimate of costs for the remainder of contract is
A.E.C. participation 84,949.12
S.R.I. participation 2,776.10
\$18,390.84

- (3) We do not expect to have any appreciable balance of funds available for financing project during period of performance.

Please disregard item 12 in our proposal for renewal of the subject contract (date March 31, 1955). I was confused in my understanding of the present budget standing.

With apologies for this confusion,

Yours sincerely,

Howard E. Skipper
Assistant Director

hee d:

REQUEST FOR RENEWAL OF CONTRACT

NO. AT-(40-1)-1038

ON

BODY RETENTION OF CARBON 14

FOR

ATOMIC ENERGY COMMISSION

DIVISION OF BIOLOGY AND MEDICINE



Southern Research Institute

Birmingham, Alabama

1111361

REQUEST FOR RENEWAL OF CONTRACT

NO. AT-(40-1)-1038

ON

BODY RETENTION OF CARBON 14

FOR

ATOMIC ENERGY COMMISSION

DIVISION OF BIOLOGY AND MEDICINE

SOUTHERN RESEARCH INSTITUTE

Birmingham, Alabama

March 31, 1955

1111362

REQUEST FOR RENEWAL OF CONTRACT
NO. AT-(40-1)-1038
ON
BODY RETENTION OF CARBON 14

In reply to the letter of February 9, 1955, from Dr. Herman M. Roth, Director, Research and Medicine Division, with regard to renewal of Contract No. AT-(40-1)-1038 on Body Retention of Carbon 14, the following information is provided.

1. Body Retention of Carbon 14.
2. Southern Research Institute, Biochemistry Division.
3. No other federal agency is supporting this program.
4. Scientific Scope: In view of the wide use of carbon-14 in laboratory studies, the increasing use of carbon-14 in human tracer studies, and certain military aspects concerning carbon-14 production on detonation of nuclear weapons, it has been considered worth-while to study the retention of this isotope in the body, and the radiation received by various tissues and organs (and areas of isotope concentration in such tissues and organs) following inhalation, ingestion or injection of carbon-14 labeled compounds.

During the past several years a rather complete study of the most urgent problem — long-term retention of $C^{14}O_3^-$ and radiation calculations in tissues and organs — has been carried out and published in accordance with the wishes of the Atomic Energy Commission:

- a. Studies on the Hazard Involved in Use of C^{14} . I. Retention of Carbon from Labeled Sodium Bicarbonate. J. Biol. Chemistry 180, 1187, 1949. Skipper, White and Bryan.
- b. Studies on the Hazard Involved in Use of C^{14} . II. The Effect of a Single Dose of C^{14} -labeled Sodium Bicarbonate on the Pattern of Deaths from Spontaneous Leukemia in Akm mice. Cancer Research 10, 362, 1950.
- c. Studies on the Hazard Involved in Use of C^{14} . III. Long-term Retention in Bone. J. Biol. Chem. 189, 159, 1951. Skipper, Nolan and Simpson.
- d. Studies on the Hazard Involved in Use of C^{14} . IV. Long-term Radiation in Bone. Manuscript in preparation for publication, March, 1955. Skipper, Simpson and Bell.

More recently efforts have been placed on the localization of C^{14} in cell fractions believed to be unusually sensitive to ionizing radiation. Results of extensive efforts in this area are summarized in the appended Progress Report.

Project AT-(40-1)-1038 has supported in part an extensive study on the relative uptake of certain C^{14} -labeled compounds into DNA and RNA of some twenty-five different animal tumors as compared to host normal tissue. This work has resulted in a manuscript which has been submitted to Cancer Research for publication. The authors of this paper are: Bennett, Skipper, Stock and Rhoads. Similar studies have been carried out in cortisonized hamsters and rats bearing different human tumors. The results of these studies will soon be submitted for publication.

It is proposed to continue the present work on incorporation of carbon-14 from various labeled compounds into genic chemicals of normal animal tissues and human tissues growing in animals. Such work should be of value in setting limits on dosage of certain classes of C^{14} -labeled compounds for human tracer studies and perhaps, more important, it is hoped that useful fundamental information on nucleic acid metabolism of human tumor tissue will be gained. Such information is of considerable fundamental importance in the area of radiation biology.

Further studies are planned in human tumor-bearing animals with the following C^{14} -labeled compounds.

C^{14} -Thymine	C^{14} -4-Amino-5-imidazolecarboxamide
C^{14} -Uracil	C^{14} -Inosinic acid
C^{14} -Orotic Acid	
C^{14} -Aspartic acid	
C^{14} -Guanylic acid	
C^{14} -Adenylic acid	
C^{14} -Thymidine	
C^{14} -Serine	

5. Scientific personnel:

Howard E. Skipper, Ph. D., Head Biochem. Div.	10-15%
Martelia Bell, B. S., Biologist	75%
Sally Johnson, B. S., Biologist	50%
Linda Simpson, B. S., Physicist	20%
Barbara Ann Mathews, B. S., Chemist	35%

6. Other personnel:

No significant amount of time will be charged by other personnel.

7. Other financial assistance: The salaries of scientific personnel of this institution are paid out of industrial, federal and institutional research contracts. Charges to each project are made from daily time sheets showing hourly distribution of effort. We have no other contracts concerned with the hazard involved in the use of carbon-14, however, we are carrying on other programs having to do with nucleic acid metabolism.

8. Materials, Equipment and Facilities: Completely equipped animal quarters, biochemical laboratories and tracer laboratories. No additional major equipment is required for these studies. Radioactive compounds will be required as expended.

9. Travel. No travel allowance is requested.

10. Budget:

1. Salaries:

Biochemist (part time)	\$ 2,000	
Biologist (part time)	3,410	
Biologist (part time)	1,660	
Physicist (part time)	840	
Chemist (part time)	<u>1,260</u>	9,170

2. Contribution to employees' retirement fund 320

3. Materials and supplies:

Radioactive compounds	600	
Glassware and animals	<u>450</u>	<u>1,050</u>

Subtotal 10,540

4. Overhead (8% of above) 843

11,383

S. R. I. overhead contribution to contract
(Difference between 79%* of direct salaries
(less vacation and sick leave) and A. E. C.
overhead of 8%) 5,991

\$ 17,374

* Experienced overhead rate 1954.

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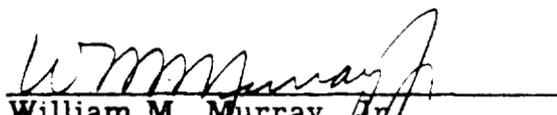
11. Amount requested \$ 11,383

The Southern Research Institute's usual research contracts bear an overhead of 100% of scientific salaries. You will note, under the budget category, that we have computed overhead differential at the rate of 79% of salaries and wages (exclusive of vacation and sick leave pay). This figure is based on rate experience in 1954 and what we assume it will be in 1955. The difference between the rate of 8% of direct costs and the rate of 79% indicates the extent of Southern Research Institute's support of the work outlined in the enclosed proposal.

12. Statement of current expenditures:

a. Contract budget	\$ 11,606
b. Expenditures thru February, 1955	9,967
c. Estimated expenditures thru June, 1955	11,606


Howard E. Skipper
Senior Investigator


William M. Murray, Jr.
Director

Birmingham, Alabama
March 31, 1955
kb (10)

Office Memorandum • UNITED STATES GOVERNMENT

TO : J. W. Culd, Jr., Assistant General Counsel DATE: May 26, 1954

FROM : R. G. Humphries, Acting Director, Contract Division

SUBJECT: RENEWAL OF CONTRACT NO. AT-(40-1)-1038 - SOUTHERN RESEARCH INSTITUTE

SYMBOL: ADA:ARB

Enclosed is an approved proposal for renewal of subject contract for a period of one year beginning July 1, 1954. This approval action is covered by Procurement Directive No. EM-54-248, dated May 12, 1954, in the amount of \$8,900.00.

Please prepare an appropriate modification to renew this contract for a period of one year, beginning July 1, 1954, and provide for the payment of \$8,900.00.



R. G. Humphries

Enclosures:

1. Request for Contract Action
2. Budget for Contract AT-(40-1)-1038
3. Resume
4. Memo fm Pearson dtd 5/12/54
5. Cy Ltr fm Pearson dtd 5/12/54
6. Cy Ltr fm Murray dtd 3/29/54
7. Request for Renewal of Contract

CC: C. S. Shoup
L. D. MacKay
Ed Ziegler, w/Encls. 1, 2 & 3
J. Nicholson

Brown:arb

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1. TO: J. R. Moore Chairman Contract Board. From: Res. & Medicine Division

It is requested that the Contract Board take the necessary action to process the following described contract action in accordance with the provisions of Bulletin OR-O&M-19:

2. Nature of Action Requested

[] Selection of New Contractor and Negotiation of Contract.

[X] Modification of Contract No. AT-(40-1)-1038 Contractor: Southern Research Institute Birmingham, Alabama

[] Review and approval of Contract, Sub-contract or Purchase Order. Number: Name:

[] Other (Explain)

3. Nature of Services to be Covered by Contract

Construction [] Architect-Engineer [] Other [X] (Explain) Research

4. Funding

Amount to be Obligated by this Contract Action : \$8,900

Source of Funds

Approved ORO Financial Plan, Quarter, Fiscal Year 19 Project No. or, Activity No. Funds to be Obligated: Allotment No. (F.Y. 19 Funds) Procurement Directive No. BM-54-248 Dated 5-12-54 Issuing Office Division of Biology and Medicine, Washington

Concurrence in Funding Statement: (signed) Chief, Budget Branch

5. Project or Activity to be Covered by Contract Action:

Location of Work: Construction Directive No. Estimated Cost of Work to be Covered by this Contract Action \$ Schedule: Date Work to Start Estimated Completion Date Description of Project or Activity:

(If more space is required use separate sheets and attach hereto:)

Bulletin OR-O&M-19

Exhibit I

Procurement Directive No. BM-54-248, 5/12/54, \$8,900.00.

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<p>6. Contract Board Docket No. _____ (To be assigned by Board Secretary)</p>	<p>7. Request Submitted By: (signed) <u>John M. Carley</u> Date: <u>5-26-54</u> Title: _____</p>
<p>8. <u>Complete Description of Services to be Furnished by Contractor:</u></p> <p>Washington designated research contract.</p> <p>Title: "Body Retention of Carbon-14"</p> <p>(If more space is required use separate sheets and attach hereto:)</p>	
<p>9. <u>Description of other changes to be covered by Modification:</u></p> <p>Renew contract for period of one year beginning July 1, 1954, with new funds in the amount of \$8,900</p> <p>(If more space is required use separate sheets and attach hereto:)</p>	
<p>10. <u>Negotiated Contracts.</u> (Show why it appears desirable to negotiate new contract or to negotiate modification to existing contract)</p> <p>Memo - P. B. Fearson to K. Kasschau, dated May 12, 1954</p> <p>(If more space is required use separate sheets and attach hereto:)</p>	
<p>11. <u>Contracts, Subcontracts, or Purchase Orders Submitted for Review and Approval:</u> (Furnish brief description of action in this space and attach pertinent documents)</p> <p>None</p>	
<p>12. <u>Disputes:</u> Attach a statement summarizing the dispute together with pertinent documents and Background Material.</p> <p>None</p>	

1111369

UNITED STATES ATOMIC ENERGY COMMISSION
DIVISION OF BIOLOGY AND MEDICINE
WASHINGTON, D. C.

DATE: MAY 1954

TO : Dr. Kenneth Kasschau, Director, Office of
Research and Medicine, Oak Ridge Operations Office
FROM : Paul B. Pearson, Chief, Biology Branch *PBP*
Division of Biology and Medicine
SUBJECT: TRANSMITTAL OF RESEARCH PROPOSAL FOR CONTRACT NEGOTIATION
SYMBOL : HMB:FBP

This letter with enclosure, in triplicate, is sent in accordance with the procedure described in a letter from the General Manager to all Managers of Operations dated January 27, 1949.

1. Institution: Southern Research Institute
2. Investigator (s): Dr. Howard E. Skipper
3. Title: Body Retention of Carbon-14.
4. () New Contract or (X) Renewal of Contract No. AT(40-1)1038
5. Duration - From: July 1, 1954 to June 30, 1955
6. AEC Technical Supervision: Biology Branch
7. Recommended Support: \$12,000.00 (less unexpended balance of \$31.00)
Authorized by Procurement Directive No. BM-54-248
Issued MAY 1954 \$ 8,900.00
Activity No. 6400
8. Other Comments:

It is understood that the contribution of the Institute to this project is not less than the difference between the overhead of 8% of direct costs and the actual overhead of 73.45% as approved by the Army Audit Agency.

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8. Comments (Continued)

9. Security Requirements:

In accordance with the provisions of GM-93 (Revised March, 1950), and the requirements of the Declassification Guide, the Division of Biology and Medicine has determined that the following security precautions should be taken in connection with the proposed research contract.

Since there is essentially zero chance that restricted data may be required or developed, no personnel security requirements should be imposed.

10. Reports: (X Reports are to be required as provided for by Memorandum Instruction of November 9, 1949, on subject "Direct Research Contract Reports".

() Special Reports Instructions are as follows:

Enclosures:	()	"A" - Proposal, dated	<u>Recd. from OakRidge</u>
	(X)	"B" - Notification letter, dated	_____
	()	"C" - Other correspondence,	_____ letters
	(X)	"D" - Procurement Directive	<u>BM-54-248</u>

Distribution:

Addressee:	Original (w encl.)	Division File:	Yellow Copy (w encl.)
	1st Copy (w encl.)		Pink Copy (w/o encl.)
	2nd Copy (w encl.)		
Program Analysis		Branch File:	White Copy (w encl.)
Branch:	White Copy (w/o encl.)		

cc: Mr. Stamwood

1111371

UNITED STATES ATOMIC ENERGY COMMISSION
DIVISION OF BIOLOGY AND MEDICINE
WASHINGTON, D. C.

TO : Dr. Kenneth Kasschau, Director, Office of Research and Medicine, Oak Ridge Operations Office
FROM : Paul B. Pearson, Chief, Biology Branch
SUBJECT: TRANSMITTAL OF RESEARCH PROPOSAL FOR CONTRACT NEGOTIATION
SYMBOL : **MB-FBP**

DATE:

This letter with enclosure, in triplicate, is sent in accordance with the procedure described in a letter from the General Manager to all Managers of Operations dated January 27, 1949.

1. Institution: **Southern Research Institute**
2. Investigator (s): **Dr. Howard E. Skipper**
3. Title: **Body Retention of Carbon-14.**
4. () New Contract or () Renewal of Contract No. AT(40-1)1838
5. Duration - From: **July 1, 1954 to June 30, 1955**
6. AEC Technical Supervision: **Biology Branch**
7. Recommended Support: **\$12,000.00 (less unexpended balance of \$3100)**
Authorized by Procurement Directive No. BM-54-248
Issued _____ ; 8,900.00
Activity No. 6400
8. Other Comments:

It is understood that the contribution of the Institute to this project is not less than the difference between the overhead of 8% of direct costs and the actual overhead of 73.45% as approved by the Army Audit Agency.

EMB:PMF

Dr. Edward E. Skipper
Assistant Director
Southern Research Institute
Birmingham 4, Alabama

Dear Dr. Skipper:

Your research proposal on "Body Retention of Carbon-14" was considered at a recent meeting of our Research Committee. I am glad to advise you that the committee approved renewal of your project for another year.

You will hear from the Oak Ridge Operations Office in the near future regarding negotiation of the renewal contract.

Sincerely yours,

Paul E. Pearson
Chief, Biology Branch
Division of Biology and Medicine

cc: William M. Murray, Jr., Director

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~~Resume~~

Studies will be performed on the chromosomal incorporation of carbon-14 from variously labeled organic compounds. ~~animals.~~ In addition, extensive investigations will be made on localization of carbon-14-labeled compounds of the nucleic acid and protein precursor types in various tissues. These studies should yield information about rates of biological processes having to do with growth in normal and neoplastic tissues.

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Budget for Contract No. AT-(40-1)-1038

For Period 7-1-54 - 6-30-55

(1) Salaries & Wages:		\$10,250.00
Biochemist, Biologists, and Physicist (all part time)	\$9,910.00	
Employees Retirement Fund	340.00	
(2) Materials & Supplies:		712.00
(3) Travel:		150.00
(4) Overhead:		
	<i>Grand Total</i>	<u>7,278.89</u> <u>\$18,390.89</u>

The Commission's contribution to the above budget will be \$12,000 including \$8,900 in new funds and the unexpended balance of \$3,100 from the previous period.

1111376

Southern Research Institute



BIRMINGHAM 5 ALABAMA

March 29, 1964

Dr. Kenneth Kasschau
U. S. Atomic Energy Commission
Research and Medicine Division
P. O. Box 8
Oak Ridge, Tennessee

Dear Dr. Kasschau:

I wish to officially endorse the attached proposal and state this Institute's willingness to accept the contract if awarded to us as requested in this proposal.

You will note that we have included overhead at 8% of direct costs. The Army Audit Agency has recently completed an overhead audit of this Institute's 1963 operations and has recommended a rate of 73.45% of direct salaries as the proper overhead rate for use during 1964 in contracts with various government agencies. I believe this is the best information we can submit to indicate the extent to which Southern Research Institute will participate in support of the work described in the enclosed proposal.

Very truly yours,

William M. Murray, Jr.
Director

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enclosures
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D-1383

REQUEST FOR RENEWAL OF CONTRACT

NO. AT-(40-1)-1038

ON

BODY RETENTION OF CARBON 14

FOR

ATOMIC ENERGY COMMISSION

DIVISION OF BIOLOGY AND MEDICINE

SOUTHERN RESEARCH INSTITUTE

BIRMINGHAM, ALABAMA

MARCH 25, 1954

T111378

101

REQUEST FOR RENEWAL OF CONTRACT
NO. AT-(40-1)-1038
ON
BODY RETENTION OF CARBON 14

In reply to the letter of March 1, 1954, from Dr. Kenneth Kasschau, Director, Research and Medicine, with regard to renewal of Contract No. AT-(40-1)-1038, on Body Retention of Carbon 14, the following information is provided.

1. Current statement of expenditures extrapolated to June 30, 1954:

Contract budget		\$ 11,379.25
Expenditures through February 28, 1954	\$ 5,084.25	
Estimated expenditures March through June, 1954	<u>3,200.00</u>	<u>8,284.25</u>
Estimated balance as of June 30, 1954		\$ 3,095.00

2. A statement of the acceptance of the proposed renewal signed by Dr. W. M. Murray, Jr., Director of Southern Research Institute, is appended.

3. Scope and present status:

(a) A manuscript on a second phase of our work on long-term radiation of bone following administration of C^{14} -bicarbonate is enclosed herewith. We have held up publication of this work until the present, pending development of bone autoradiograms which took long periods for development.

(b) During the past year much effort has been expended in obtaining information on radiation received by chromosomal constituents (nucleic acids) from incorporated C^{14} following injection of known precursors of nucleic acids. This sort of information has been obtained in certain animal tissues and in human sarcoma growing rapidly in hamsters. It is hoped that such information will be of value to the "Committee on Human Applications" in future decisions as to the "allowable amounts" of labeled compounds for human experimentation. Also a considerable amount of effort has been directed toward making autoradiograms of giant chromosomes following administration of labeled nucleic acid precursors to *Drosophila*. This latter effort has not been too rewarding and we propose to cease any considerable effort in this field unless the AEC has unusual interest in this area.

PLAN OF APPROACH

We consider that with the completion of the work described in the enclosed manuscript, our efforts on one phase of the problem of the hazard involved in use of C^{14} is completed. Two separate studies on the radiation being received by the "active area" of bone (areas of deposition in the shaft) at extended periods after administration of $NaHC^{14}O_3$ have been carried out. The results obtained were in good agreement and should be helpful in assessing "allowable limits."

It is suggested that we turn our attention to chromosomal (nucleic acid) incorporation of carbon 14 from variously labeled organic compounds in animal tissues and human sarcoma grown in animals. This effort is to occupy only a relatively small portion of our time.

It is proposed that we undertake a rather extensive effort on localization (in short term experiments) of C^{14} -labeled compounds of the nucleic acid and protein precursor types in various tissues including human sarcoma in hamsters. The objective of such work would be to attempt by tissue autoradiogram techniques to learn more about rates of biological processes having to do with growth in areas of various tissues, both normal and neoplastic. Such information would be useful in determination of radiation dosage in rep/day in "active areas" of tissues following administration of C^{14} -labeled organic compounds. Also it is hoped that such data would help to explain the lack of uniformity of the cytotoxic effects of certain metabolite antagonists. Labeled compounds available for such studies in this laboratory are listed below:

C^{14} -formate	C^{14} -thymine
C^{14} -glycine	C^{14} -thymidine
C^{14} -serine	C^{14} -uracil
C^{14} -adenine	S^{35} -6-mercaptapurine
C^{14} -guanine	C^{14} -nitrogen mustard
C^{14} -2, 6-diaminopurine	C^{14} -folic acid
C^{14} -hypoxanthine	C^{14} -aminopterin
C^{14} -xanthine	C^{14} -4-amino-5-imidazole carboxamide

BUDGET JUNE 30, 1954 TO JUNE 30, 1955

In compliance with a letter from the Division of Biology and Medicine, we have husbanded funds as carefully as possible this year and it appears that we will have approximately \$3,100.00 of our 1953 budget remaining at the end of the current contract period. It is suggested that a budget of \$12,000.00 (\$8,900.00 plus the \$3,100.00 unexpended balance of this year's funds) would allow for the proposed effort.

A breakdown of the budget is provided below:

(1) Salaries:

Biochemist (part time)	\$ 4,500.00	
Biologist (part time)	3,410.00	
Biologist (part time)	1,000.00	
Physicist (part time)	<u>1,000.00</u>	\$ 9,910.00

(2) Contribution to employees' retirement fund 340.00

(3) Materials and supplies:

Radioactive compounds	300.00	
Glassware and animals	<u>412.00</u>	712.00

(4) Travel 150.00

Subtotal 11,112.00

(5) Overhead (8%) ~~888.00~~

Total \$ 12,000.00

Birmingham, Alabama
March 25, 1954
mw (10)

Office Memorandum • UNITED STATES GOVERNMENT

TO J. W. Culp, Jr., Assistant General Counsel

DATE: May 25, 1953

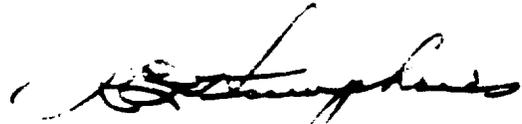
FROM R. G. Humphries, Assistant Director, Contract Division

SUBJECT: RENEWAL OF CONTRACT NO. AT-(40-1)-1038 - SOUTHERN RESEARCH INSTITUTE

SYMBOL: AD:ARB

Enclosed is an approved proposal for renewal of subject contract for one year beginning July 1, 1953. This approval action is covered by Procurement Directive No. EM-53-280, dated May 14, 1953, in the amount of \$7,238.00.

It is requested that an appropriate modification be prepared to renew this contract for a period of one year beginning July 1, 1953, at a level of \$6,858.00, in accordance with the budget in that amount attached to the Request for Contract Action.



R. G. Humphries

Enclosures:

1. Request for Contract Action.
2. Memo fm Wilbur dtd 5/14/53.
3. Cy Ltr fm Butts dtd 5/14/53.
4. Budget.
5. Resume.
6. Cy Ltr fm Skipper dtd 4/28/53.
7. Cy Ltr fm Murray dtd 4/28/53, w/Proposal.

CC: C. S. Shoup
L. D. Mackay
Ed Ziegler, w/Encl. 1
J. Nicholson

/arb

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1111382

1. **John R. Moore, Director** Chairman
TO: **Contract Division** Contract Board.

From: Research & Medicine Division

It is requested that the Contract Board take the necessary action to process the following described contract action in accordance with the provisions of Bulletin OR-O&M-19:

2. Nature of Action Requested

Selection of New Contractor and Negotiation of Contract.

Modification of Contract No. AT-(40-1)-1038

Contractor: Southern Research Institute Birmingham, Alabama

Review and approval of Contract, Sub-contract or Purchase Order.

Other (Explain) _____

Number: _____
Name: _____

3. Nature of Services to be Covered by Contract

Construction

Architect-Engineer

Other

(Explain) **Research**

4. Funding

Amount to be Obligated by this Contract Action \$ 6,858.00

Source of Funds

Approved ORO Financial Plan, _____ Quarter, Fiscal Year 19__

Project No. _____ or, Activity No. _____

Funds to be Obligated: Allotment No. _____ (F.Y. 19__ Funds)

Procurement Directive No. BM-53-280 Dated 5-14-53

Issuing Office Division of Biology and Medicine, Washington

Concurrence in Funding Statement: (signed) _____

Chief, Budget Branch

5. Project or Activity to be Covered by Contract Action:

Location of Work: _____ Construction Directive No. _____

Estimated Cost of Work to be Covered by this Contract Action \$ _____

Schedule: Date Work to Start _____ Estimated Completion Date _____

Description of Project or Activity:

(If more space is required use separate sheets and attach hereto:)

Bulletin OR-O&M-19

Exhibit I

*Approved by
in Birmingham 5/14/53
p/r ccc*

1111383

6. Contract Board Docket
No. _____
(To be assigned by
Board Secretary)

7. Request Submitted By: (Signed) _____
Date: _____ Title: _____

8. Complete Description of Services to be Furnished by Contractor:

Washington designated research contract

Title: Body Retention of Carbon-14

(If more space is required use separate sheets and attach hereto:)

9. Description of other changes to be covered by Modification:

Renewal of Contract for period of one year, beginning July 1, 1953

(If more space is required use separate sheets and attach hereto:)

10. Negotiated Contracts. (Show why it appears desirable to negotiate new contract or to negotiate modification to existing contract)

Memo K. M. Wilbur to Kenneth Kasschau to May 14, 1953

(If more space is required use separate sheets and attach hereto:)

11. Contracts, Subcontracts, or Purchase Orders Submitted for Review and Approval: (Furnish brief description of action in this space and attach pertinent documents)

None

12. Disputes:

Attach a statement summarizing the dispute together with pertinent documents and Background Material.

None

UNITED STATES ATOMIC ENERGY COMMISSION
DIVISION OF BIOLOGY AND MEDICINE
WASHINGTON, D. C.

DATE: _____

TO : Dr. Kenneth Kasschau, Director, Office of
Research and Medicine, Oak Ridge Operations Office
FROM : Karl M. Wilbur, Physiologist, Biology Br.
Division of Biology and Medicine
SUBJECT: TRANSMITTAL OF RESEARCH PROPOSAL FOR CONTRACT NEGOTIATION
SYMBOL : BMB:KMW

This letter with enclosure, in triplicate, is sent in accordance with the procedure described in a letter from the General Manager to all Managers of Operations dated January 27, 1949.

1. Institution: Southern Research Institute
2. Investigator (s): Dr. Howard E. Skipper
3. Title: Body Retention of Carbon-14
4. () New Contract or Renewal of Contract No. AT(40-1)1038
5. Duration - From: July 1, 1953 to June 30, 1954
6. AEC Technical Supervision: Biology Branch
7. Recommended Support: \$11,988, includes 8% overhead (less \$4750 unexpended balance)
Authorized by Procurement Directive No. BM-53-280
Issued _____ \$ 7,238.00
Activity No. 6400
8. Other Comments:

This project was approved for \$11,988, less the unexpended balance of approximately \$4750.

1111385

BMB:JSB

MAY 14 '53

Dr. Howard E. Skipper
Assistant Director
Southern Research Institute
Birmingham 4, Alabama

Dear Dr. Skipper:

It is a pleasure to tell you that the Research Committee has reviewed your proposal with considerable interest and have approved renewal for another year. We hope that you may have continued success with your program during the coming year.

You may expect to hear from the Oak Ridge Operations Office within the near future regarding negotiations of the contract.

Sincerely yours,

Joseph S. Metts
Biochemist, Biology Branch
Division of Biology and Medicine

cc: Dr. William M. Murray, Jr., Director

1111387

Southern Research Institute
Birmingham, Ala.

April 22, 1953.

Dr. Kenneth Kasschau
Director, Research and Medicine Division
U. S. Atomic Energy Commission
Post Office Box 5
Oak Ridge, Tennessee

Dear Dr. Kasschau:

In reply to your letter of April 8, 1953 regarding renewal of Contract No. AT-(40-1)-1038, "Body Retention of Carbon 14," the following documents are enclosed:

- (1) Request for renewal of Contract No. AT-(40-1)-1038, which includes the information requested on budgetary matters, approach, etc.
- (2) A statement of acceptance of the renewed contract signed by Dr. W. M. Murray, Jr., Director of Southern Research Institute.
- (3) Status Report No. 17.
- (4) A reprint from Nucleonics summarizing a phase of our past efforts.

As you will note, we are asking for a cut in our budget. I assume that this will not be disagreeable to you.

If further information or details are desired, please do not hesitate to call on us.

Yours very truly,

Howard E. Skipper
Howard E. Skipper
Assistant Director

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APR 29 1953

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Southern Research Institute
Birmingham, Ala.

April 28, 1953

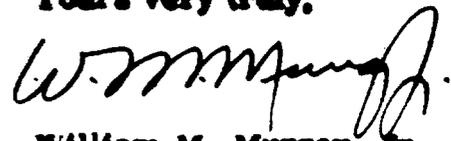
**Dr. Kenneth Kasehan
Director, Research and Medicine Division
U. S. Atomic Energy Commission
Post Office Box E
Oak Ridge, Tennessee**

Dear Dr. Kasehan:

In accord with item 2 of your letter of April 8 regarding renewal of Contract No. AT-(40-1)-1038, I should like to state that should the Atomic Energy Commission decide to renew the above-mentioned contract for the period July 1, 1953 to July 1, 1954, this institution would be pleased to accept the subject research contract.

It has always been a pleasure for Southern Research Institute to participate in the Atomic Energy Commission's research program.

Yours very truly,



**William M. Murray, Jr.
Director**

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REQUEST FOR RENEWAL OF CONTRACT

NO. AT-(40-1)-1038

- ON

BODY RETENTION OF CARBON 14

FOR

ATOMIC ENERGY COMMISSION

DIVISION OF BIOLOGY AND MEDICINE

SOUTHERN RESEARCH INSTITUTE

BIRMINGHAM, ALABAMA

APRIL 28, 1958

PROPOSAL NO. 584

1111390

**REQUEST FOR RENEWAL OF CONTRACT
NO. AT-(40-1)-1038
ON
BODY RETENTION OF CARBON 14**

In reply to the letter of April 8 from Dr. Kenneth Kasechau, Director, Research and Medicine Division, with regards to renewal of Contract No. AT-(40-1)-1038 on Body Retention of Carbon 14, the following information is provided.

1. Current statement of expenditures extrapolated to June 30, 1953.

Contract budget		\$15, 222.00
Expenditures through March, 1953	2, 121.00	
Estimated expenditures April, May, June, 1953	<u>2, 400.00</u>	
		<u>10, 583.00</u>
Estimated balance as of June 30, 1953		\$ 4, 739.00

2. A statement of acceptance of the proposed renewal signed by Dr. W. M. Murray, Jr., Director of the Institute, is appended.

3. A progress report, No. 17, is enclosed herewith.

4. Scope and present status:

- a. Extensive efforts have been reported on the long-term retention of C^{14} , the localization of carbon 14, and the roentgen-equivalent physical received by the "active" area of mouse bones at periods up to two years following a single injection of $NaHC^{14}O_3$. The results of these experiments have been reported in detail in progress reports and in scientific publications. A summary of some of this work is contained in a paper "The Hazards Involved in the Use of Carbon 14," *Nucleonics* 10, 40-44, 1952 by Howard E. Skipper (reprint appended). In addition**

RESEARCH INSTITUTE

¹⁴C O₂ retention studies have been repeated and extended to approximately two years with appropriate radiation calculations (see Progress Report No. 17).

Much effort has been expended in the development of techniques which might allow for preparation of autoradiograms of giant chromosomes from *Drosophila* following injection or feeding of carbon 14 labeled compounds. It is believed that technical difficulties encountered in this effort have been overcome and that the procedure can be used to advance our knowledge of chromosome metabolism, the possible site of radiation damage.

Retention and metabolism of a number of organic compounds, the most recent of which is 2-labeled folic acid (crystalline) have been studied.

PLAN OF APPROACH

It is planned to continue our studies on distribution, retention and metabolism of carbon 14 labeled compounds in the mouse. Such information is needed in all efforts to estimate allowable levels of C¹⁴-labeled organic compounds which might be used in human experimentation. A rather large number of C¹⁴-labeled nucleic acid precursors and antagonists are being prepared and procured for other projects in this laboratory. These will be employed in retention studies. Among the compounds are all of the nucleic acid bases, the known "de novo" precursors and five purine antagonists.

It is also planned to use the chromosome-autoradiogram procedures developed under grant No. AT-(49-1)-1038 to study "in vivo" chromosome metabolism with the view to providing fundamental information which is of possible interest to the problem of radiation damage. This information will be correlated with data obtained in a large program on nucleic acid metabolism in progress at this institution. There are large amounts of data suggesting that the site of action of a number of temporary anticancer agents, including ionizing radiation, may be at the gene-chemical level.

BUDGET - JUNE 30, 1953 TO JUNE 30, 1954

The budget for 1953 was larger than will be required for the proposed effort for the coming year. It is suggested that the budget for 1953 be cut to \$12,000.00. Since approximately \$5,000.00 will remain unspent at the end of the current fiscal year, it is suggested that, should the sponsor desire to continue this work, an amount of \$7,000.00 plus the unspent \$5,000.00 making a total of \$12,000.00 for the coming year, would be adequate.

A breakdown of the budget is provided below:

(1) Salaries

Biochemist (part-time)	\$4,500.00	
Biologist (full-time)	3,410.00	
Biologist (part-time)	1,000.00	
Physicist (part-time)	1,000.00	
	<u>\$9,910.00</u>	\$9,910.00

(2) Contribution to Employees Retirement Fund		340.00
-----------------------------------------------	--	--------

(3) Materials and Supplies		
Radioactive compounds	300.00	
Glassware and animals	400.00	
Total Materials and Supplies		700.00

(4) Travel		150.00
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Sub-total		11,100.00
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(5) Overhead (8%)		<u>888.00</u>
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\$11,988.00

Birmingham, Alabama
April 28, 1953
Proposal No. 384
S) ed

1111393

The Hazard Involved in the Use of Carbon-14

By HOWARD E. SKIPPER

*Southern Research Institute
Birmingham, Alabama*

Reprinted from NUCLEONICS, February, 1952
Vol. 10, No. 2, Pages 40-44

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McGraw-Hill Publishing Company, Inc.
330 West 42nd Street
New York 36, New York

1111394

TABLE 1—Rate of Excretion of C^{14} Following Injection of $NaHC^{14}O_3$ into Mice (18 μc)

Period	Specific activity of exhaled $C^{14}O_2$ $\mu c/ml \cdot l$	% of total injected activity	Cumulative % of total activity
0-10 min	13,770	66	66
10-20 "	3,603	16	82
20-30 "	1,750	6.2	88.2
30-60 "	500	4.6	92.8
1-2 hr	84	1.3	94.1
2-4 "	24.1	0.65	94.75
4-6 "	11.2	0.26	95.01
6-12 "	5.6	0.44	95.45
12-24 "	4.8	0.59	96.09
24-hr composite urine sample	115.1	1.35	97.39

TABLE 2—Specific Activities of Tissues at Periods After Injection of $NaHC^{14}O_3$ (18 μc)

Period after injection	Blood	Liver	Brain	Jejunum	Long bone	Lung shaft
24 hr	1.1	1.1	0.26	2.16	1.89	1.08
1 wk	0.25	0.48	0.18	0.18	0.33
1 mo	0.11	0.02	0.13	0.02	0.11	0.25
3 mo	0.10	0.07	0.03	<0.009	0.09	0.15
5-3 mo	<0.009	0.01	<0.009	0.04	0.06
8-7 mo	<0.009	<0.009	<0.009	0.07	0.12
10-2 mo	0.04	0.05
10-7 mo	0.06	0.10
12-7 mo	0.03	0.04

The Hazard Involved in

The most popular of the radioisotopes, carbon-14 is employed in hundreds of laboratories. When used with caution and as an experimental tool, it is considered nonhazardous. Evidence and new data to support this conclusion are reviewed here, together with a discussion on the nature of the hazard

By HOWARD E. SKIPPER
Southern Research Institute
Birmingham, Alabama

WHEN CARBON-14 first became available from the Atomic Energy Commission, it was considered one of the more dangerous isotopes because of its long half-life (about 5,500 years) and because of the well-known entrance of carbon from carbon dioxide into the organic and inorganic metabolic pathways. This picture has gradually changed.

When employed at usual experimental levels with reasonable precautions, C^{14} is now generally assumed to be nonhazardous. In support of this assumption, this paper presents certain

basic experimental evidence that seems to bear on the question of the hazard involved in laboratory use of C^{14} .

The two C^{14} -containing substances with which the laboratory worker comes into contact most frequently (prior to dilutions with inactive carbon) are C^{14} -carbon dioxide and C^{14} -barium carbonate. Obviously, the former can be inhaled as such, and barium carbonate can, when dry, be inhaled as a dust or aerosol. It is also apparent that in many C^{14} -labeled organic compounds the biological fate of the isotope is oxidation to $C^{14}O_2$.

To assess the most obvious hazards involved in the use of C^{14} , one must know the:

1. Degree of exchange of inspired $C^{14}O_2$ with blood bicarbonate at sites of blood aeration in the lungs.
2. Retention of bicarbonate carbon in the blood and various tissues (this depends on the exchange and on the rates of metabolism, i.e., CO_2 fixation and turnover).
3. Degree of localization of the isotope within certain more-or-less active cells within various tissues.
4. Stability of $BaC^{14}O_3$ particulates retained by the lung on inspiration of $BaC^{14}O_3$ aerosol.
5. Inherent carcinogenicity of C^{14} due to the physical characteristics of its atomic disintegration.

Carbon Dioxide and Bicarbonate

Brues and Buchanan (1) have provided information on the retention of C^{14} by fixation of inhaled $C^{14}O_2$ gas. They observed that the lungs absorb $C^{14}O_2$ very efficiently in spite of the mass movement of CO_2 in the opposite direction. It appears that the amount of $C^{14}O_2$ absorbed to reach equilibrium with the blood bicarbonate depends on the specific activity of the alveolar atmosphere and thus is largely independent of the CO_2 concentration in

1111395

TABLE 3 Per Cent of the Total Injected C¹⁴ Retained at Various Periods

Period of	Total		% of retained activity in skeleton
	body	skeleton	
24 hr	1.4	0.25	18
48 hr	0.94	0.19	20
1 wk	0.62	0.09	15
2 wk	0.20	0.07	35
1 mo	0.13	0.03	23
3 mo	0.12	0.03	25
5.3 mo	<0.02	0.01	>50
8.7 mo	<0.04	0.03	>75
10.3 mo	0.02
12.7 mo	0.01

TABLE 4 - Total Body and Skeleton Radiation Calculations on NaHC¹⁴O₃ Injected Mice

Period	Body radiation in rads per gram per period	Skeleton radiation in rads per gram per period
0-24 hr	0.11	0.07
1-2 days	0.027	0.033
2-7 days	0.022	0.021
1-2 wk	0.009	0.016
2-4 wk	0.004	0.008
1-3 mo	0.003	0.004
5.8-6.2 mo	0.004
8.7-10.2 mo	0.004
10.8-12.2 mo	0.002

NOTE: Maximum tolerated level estimated at 0.05 r per day

the Use of Carbon-14

the inhaled air. Brues and Buchanan state that "in general, following exposure, C¹⁴ is removed at a rate comparable to that of its uptake, and the same is probably true of its incorporation into compounds in a steady state. One probable exception exists in the case of growing tissue and, of course, where exposure is continuous over a long period."

It has been adequately demonstrated that the major fraction of the C¹⁴ from labeled bicarbonate injected into animals is expired within a short time (about 65% in 10 min; >90% in 4 hr) (2, 3, 4). Table 1 gives certain data we have obtained on this subject (4).

This rapid initial turnover of the greater portions of the C¹⁴ in blood bicarbonate is consoling. However, it seems important to know something of the degree and duration of tissue exposure to C¹⁴ following the intake of compounds labeled with this isotope, especially bicarbonate. The degree and duration of exposure of tissues to the β-particles from C¹⁴ are dependent on concentration and turnover, both of which are in turn dependent on the carbon-utilizing metabolic system involved and its dynamic state. Tissue exposure data can best be obtained by following the quantitative gross dis-

tribution and turnover of C¹⁴ in animals for extended periods.

Bloom, Curtis, and McLean (5) showed by autoradiographic technique that the bones of rats injected with BaC¹⁴O₃ (75 to 100 μc) retained activity at 16 weeks. Sections of liver and kidney gave fairly intense autoradiographs at 3 days and 2 weeks, but were negative after longer intervals.

Armstrong, Schubert, and Lindenbaum (2) in studies of the distribution of radiocarbon administered as carbonate observed retention in soft tissues, bones, and teeth at 6 days and in certain cellular constituents at 40 days.

We have carried out certain experiments designed to obtain quantitative data on C¹⁴O₂ fixation, from which tissue, organ, and total body exposure can be estimated (4, 6). To be able to carry on these experiments over long periods, and to avoid use of excessive amounts of C¹⁴, it was necessary to employ extremely sensitive counting techniques (7) and small animals.

Using the principles described by Miller (8), Dr. Locke White, Jr., of our Physics and Physical Chemistry Division, constructed a gas-phase apparatus, which has allowed us to determine rather accurately the C¹⁴ content of mouse bones one year after injection of 18 μc of NaHC¹⁴O₃. Because of

the excellent geometry of this Geiger tube (7), almost all disintegrations are recorded, allowing for estimates of absolute activity of C¹⁴O₂ from oxidized tissue samples.

Adult mice were injected intraperitoneally with 18 μc of NaHC¹⁴O₃ (2.5 mg of sodium bicarbonate with a specific activity of 600 mc/mole of carbon). This is approximately a 50-mc man-equivalent. The specific activities of a number of samples of tissues, blood, and bone were then determined at 24 and 48 hours, 1 and 2 weeks, and 1, 3, 4, 5, and 9 months (4, 6). From these specific activities and knowledge of the tissue weights, estimates of the total activity in each tissue sample were made.

After 3 months, none of the soft tissue retained significant quantities, so that later assays were limited to bone. Bone activities have now been followed as long as 12.7 months (7). Certain of the data on this subject have been selected for illustrative purposes in Table 2.

Calculations on the per cent of the total injected C¹⁴ from NaHC¹⁴O₃ retained in the body and the skeleton at various periods are presented in Table 3.

Knowing the approximate amount of C¹⁴ in the body or a portion thereof

Bone Section: 24 Hours



FIG. 1. Bone section and autoradiograph from a mouse at 24 hours after injection of 18 μc of C^{14} -bicarbonate ("No-Screen" X-ray film exposed for 30 days prior to development)

Bone Section: 6 Months



FIG. 2. Bone section and autoradiograph from a mouse at 6 months after injection of 18 μc of C^{14} -bicarbonate (X-ray film exposed for 5 months)

and the average energy of radiation of this isotope, it is possible to convert tissue or total-body activities, integrated into roentgen units.

Assuming equal distribution in the body and in the skeleton, radiation calculations have been made and are presented in Table 4. The latest estimates on the maximum permissible dose for any tissue is 0.3 r/wk (9), or 0.05 r/day.

Since it is well known that C^{14} emits a very soft β -ray that traverses only several cell diameters, such data as have been presented in Table 4 do not present a picture of the maximum radiation in limited areas of slow turnover. It has been considered worthwhile to attempt to estimate the degree of radiation possible in areas of the bone where, as indicated by autoradiographs, C^{14} retention is highest.

Autoradiographs were prepared by embedding bones of $\text{NaHC}^{14}\text{O}_3$ -in-

jected mice in plastic, and then grinding about half way through the bone. These sagittal sections were then placed in close contact with "No-Screen" X-ray film for about 4 or 5 months (10). Figures 1 and 2 show typical autoradiographs made from bones taken at various periods after injection of 18 μc of C^{14} bicarbonate.

As can be seen from the righthand picture in Fig. 2, the "active" area (after 2 weeks) can be considered roughly as a cylinder with a wall thickness the average width of the parallel black lines. Hence the active volume can be approximated from actual measurements by multiplying the average length of the lines by the average width and multiplying that by πD , where D is the diameter of the hypothetical radioactive cylinder containing the preponderance of C^{14} .

Such measurements have been made with a calibrated filar micrometer on

bone autoradiographs from mice sacrificed 2 weeks, and 5.8, 6.2, and 6.3 months after injection with C^{14} -bicarbonate 18 μc . Based on total activity determination on corresponding bones in the same mouse, calculations have been made on total activity per cubic millimeter of "active" bone and the approximate degree of radiation (r/day) received by the "active" bone. These values are summarized in Table 5.

Such calculations are, of course, approximate, and it is fully appreciated that the assumption that all of the active carbon in the bone is contained in the so-called active volume is not a valid one. It, however, is a conservative one, and the calculations given in Table 5 provide radiation values which are believed to be of the correct order.

These calculations imply that for the period of 1 to 2 weeks after injection of

TABLE 5—Radiation Received per Day by "Active" Volume of Bone at Periods After Injection of $\text{NaHC}^{14}\text{O}_3$ (18 μc)

Period	Volume of active bone (mm^3)	Activity per unit "active" volume ($\text{m}\mu\text{c}/\text{mm}^3$)	Average radiation in active bone (r/day)
1-2 wk	8.2	0.04	0.16
5.3-5.8 mo	3.7	0.03	0.04
5.8-6.2 mo	5.4	0.02	0.04
6.2-6.3 mo	6.6	0.01	0.03

TABLE 6—Particle Size Distribution of a $\text{BaC}^{14}\text{O}_3$ Aerosol Measured by Microscopic Inspection

Diameter (microns)	% of particles
<2	70.5
2-3	20.8
3-4	6.3
4-5	1.9
>5	0.5
	100.0

TABLE 7—Specific Activities of Lungs after Exposure to an Aerosol of BaC¹⁴O₃

Expt. No.	Specific activity of BaC ¹⁴ O ₃	Period of exposure, min.	Time of sacrifice	Specific activities, $\mu\text{Ci/gm. C}$			
				Lungs	Spleen	Kidneys	Liver
1	6,700	66	Immediate	0.03			
			24 hr	<0.005			
			48 hr	<0.005			
			7 days	<0.005			
2	20,000	120	Immediate	0.02			
			1 hr	0.03			
			3 hr	<0.005			
3	200,000	30	Immediate	0.04	<0.005	0.01	<0.005
			Immediate	0.05			
			24 hr	0.016			
			24 hr	<0.005			

NOTE: All activities are on individual mice sacrificed at indicated intervals.

TABLE 8—Specific Activities of Exhaled CO₂ from Mice Exposed to a BaC¹⁴O₃ Aerosol

Period, days	Specific activity of exhaled CO ₂ , $\mu\text{Ci/gm. C}$
0-1	0.31
1-2	0.06
2-5	0.11
5-6	0.09
6-7	<0.01

TABLE 9—Effects of Injection of NaHC¹⁴O₃ (18 μC) on the Pattern of Death from Spontaneous Leukemia

	Controls	NaHC ¹⁴ O ₃
Mortality	13/13	15/15
Average age at leukemic death	306 days	308 days
Standard deviation from mean	51.2 days	79.0 days

18 μC of C¹⁴-bicarbonate into mice, certain bone shaft cells were receiving about 0.16 r/day, but that after 4 months this "active" area bone was receiving about 0.04 r/day. Since the recommended maximum permissible dose for any tissue is 0.05 r/day, it would appear that an 18- μC dose for a 25-gm mouse (a 50-mc man-equivalent) produces localized radiation which is, for a time, above the allowable level.

Barium Carbonate

The possible hazard from C¹⁴-barium carbonate dust inhaled by the laboratory worker has been a source of considerable concern.

Govaerts (10) has studied the rate of elimination of C¹⁴ administered as BaC¹⁴O₃ by intraperitoneal injections in rats. He reported that "in preliminary experiments, 30-60 minutes after injecting the barium carbonate, C¹⁴ was rapidly fixed by the lungs and no significant activity was found in soft tissues." The excretion of the C¹⁴ by the lungs was very rapid, about 45% of the total injected C¹⁴ being expired during the first hour and about 85% in 12 hours. Approximately 88-99% of the total activity was expired and excreted (apparently in about 2 days).

This author cannot understand why Govaerts was unable to demonstrate significant C¹⁴O₂ fixation in all tissues after BaC¹⁴O₃ injections since, in his experiments, there was obvious whole-

sale exchange with body carbonate.

Using a procedure suggested by Dr. H. D. Landahl of the University of Chicago Toxicity Laboratory, we have been able to set up a BaC¹⁴O₃ aerosol of primary particles for animal exposures. A small amount of BaC¹⁴O₃ was placed in a 250-ml three-necked reaction flask with an appropriate inlet and outlet. The inlet tube was drawn down to about a 1/2 liter, min orifice (under 15 psi) and inserted through a rubber stopper so that the lower end was about 1 in. from the bottom of the flask. Air pressure was then applied (15 psi) to the affluent of the system while gently but rapidly tapping on the flask with a rubber-covered striker from an electric bell. The larger particles were removed in additional flasks.

A BaC¹⁴O₃ aerosol, so produced, appeared to be very fine and could be detected by means of a Tyndall beam an hour after it was set up in a glass desiccator used as an animal chamber. Its particle-size distribution was estimated by microscopic inspection (using a filar micrometer) of settled particulates; see Table 6.

Mice have been exposed to BaC¹⁴O₃ aerosols of different specific activities and sacrificed for tissue C¹⁴-assays at various intervals. The results (11) are presented in Table 7.

In addition, mice were exposed to a BaC¹⁴O₃ aerosol, removed from the exposure chamber, and placed in a

metabolism chamber where exhaled CO₂ was collected for one week. The specific activities of the exhaled C¹⁴O₂ are given in Table 8.

These findings seem to confirm those on injected BaC¹⁴O₃ which suggest that an inhaled particle of barium carbonate exchanges its carbon atoms fairly rapidly with body pool carbonate and that inhaled BaC¹⁴O₃ is perhaps not as dangerous as might have been expected on the basis of its insolubility.

Effects of C¹⁴ on Mice with Spontaneous Leukemia

It seems important, along with studies on the absorption, retention, distribution, and turnover of C¹⁴ when administered in various chemical states, to make some direct observations on the carcinogenic activity of this radioisotope.

Preliminary investigations have been reported (12) on the failure of a single dose of NaHC¹⁴O₃ (18 μC , a 50-mc-man equivalent) to affect the pattern of deaths of Akm mice from spontaneous leukemia. This strain of animal, because of inbreeding, develops spontaneous leukemia in almost 100% of cases at about 9-10 months of age. When young Akm mice were injected with NaHC¹⁴O₃ and set aside to await development and death from spontaneous leukemia, it was observed that their pattern of deaths was no different from litter-mate controls injected with nonradioactive bicarbonate;

the results of this study are summarized in Table 9.

Similar studies have now been carried out with low levels (1.4 μc) of C^{14} -labeled 2,6-diaminopurine, which acts as a precursor of chromosome guanine, and with high levels of C^{14} -formate (250 μc , a 700-mc man-equivalent), which acts as a precursor of the 2- and 8-carbon atoms of deoxyribose nucleic and ribose nucleic acid purines and the methyl carbon atom of thymine. The effects of these C^{14} -labeled compounds on the pattern of deaths from spontaneous leukemia in mice was likewise insignificant (11).

One of the more sensitive methods of detecting radiation effects in animals is by means of white blood counts. Leukemic white-blood-cell production is apparently more sensitive to radiation than normal hematopoiesis. We have injected leukemic mice with 100 μc of $\text{HC}^{14}\text{OONa}$ and observed no depression of the high white blood count (13). Also it was observed that 200 μc of $\text{HC}^{14}\text{OONa}$ (a 500-mc man-equivalent) had no effect on the life span of mice with transplanted Ak-4 leukemia (13).

These experiments suggest that C^{14} (even when temporarily incorporated into the chromosome, which is the case with isotopic formate) at the levels

indicated has no profound cytotoxic activity.

Conclusions

In summary, we might make the following guarded statements:

1. Inhaled C^{14}O_2 exchanges with blood bicarbonate fairly extensively, but by the same token is not retained in the body to any great extent. The blood bicarbonate is turned over largely in a matter of minutes.

2. Of the inhaled C^{14}O_2 , a small amount is fixed in body tissues. The turnover of this C^{14} is dependent on many metabolic processes. The turnover of C^{14} from bicarbonate in the bone is much slower than that in the soft tissues.

3. After injection of 18 μc of C^{14} -bicarbonate into mice (a 50-mc man-equivalent), the body and the skeleton as a whole receive greater than the maximum permissible level of 0.05 r/day for only about 24 hours. However, certain areas in the bone are receiving greater than this amount for several months.

4. The carbonate moiety of $\text{BaC}^{14}\text{O}_3$ absorbed in the lungs does not remain as such, but turns over very rapidly by exchange with inactive CO_2 in the moist atmosphere of the lungs.

5. Fairly high levels (a 50-mc man-

equivalent of $\text{NaHC}^{14}\text{O}_3$ or a 700-mc man-equivalent of $\text{HC}^{14}\text{OONa}$) have shown no effects on the pattern of deaths from spontaneous leukemia in AKM strain mice, nor have indications of toxic action been observed on administration of C^{14} at these levels.

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CONTRACT NO. AT-(40-1)-1038

SOUTHERN RESEARCH INSTITUTE
BIRMINGHAM, ALABAMA

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STUDIES ON THE HAZARD INVOLVED IN USE OF C¹⁴

LONG-TERM BONE RADIATION

Earlier studies have demonstrated that at one year after administration of 18 microcuries of C¹⁴ bicarbonate to mice (a 50-millicurie man-equivalent), the whole long bones were receiving an average of approximately 0.003 roentgen-equivalent-physical (rep) daily (1). In these experiments the average whole bone radiation dropped below the suggested maximum tolerated radiation for man (0.04 rep/day) after several days, but autoradiograms showed that there was definite localization in the bone shaft and it could be estimated that the average radiation in the "active area" fell to 0.04 rep/day at about six months after a single injection of 18 μ c of NaHC¹⁴O₃.

Since such observations seemed rather pertinent to the problem of possible C¹⁴ hazard, both to the laboratory worker and to patients injected with C¹⁴-labeled compounds which are degraded to carbon dioxide, it was considered worth while to repeat these studies using higher levels of C¹⁴ and longer periods of observation.

EXPERIMENTAL

The techniques employed for injection of NaHC¹⁴O₃, gas-phase counting of bone activity, preparation of bone autoradiograms, and calculation of the radiation received by the "active area" of the bone have already been described (1). In the present experiments adult, 3-month-old mice of the CFW strain were employed. All mice were injected intraperitoneally with 100 μ c of NaHC¹⁴O₃, and single animals were sacrificed at periods up to 22 months. A femur and a humerus from each mouse were usually excised for total C¹⁴ assay and corresponding bones were autoradiogrammed on Ne-Screen X-ray film.

It is pertinent to mention that the gas-phase counting system employed in all of this work (2) has been calibrated against a National Bureau of Standards C¹⁴ Beta-ray Standard and shown to be in agreement within 4% on an absolute basis.

A comparison of the earlier data (1) with the present observations regarding the specific activities of bone at extended periods after a single injection of NaHC¹⁴O₃, are presented in Table I.

Using autoradiograms prepared from bones obtained in these experiments the "active areas" were measured and the active volumes calculated (1). Based on total activity determinations in corresponding bones in the same mouse, calculations have been made with regard to the total activity

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in 6 mm³ of "active" bone and the degree of radiation (roentgen-equivalent physical per day) in the "active" bone. These values are summarized along with previously obtained data in Table II and are plotted in Figure 1.

DISCUSSION

It can be seen from the data presented in Table I that the specific activity of bones from mice injected with 100 μ c of $\text{NaH}^{14}\text{CO}_3$ drop continuously with time until at 23 months the activity is extremely low (0.03 μ c/mole of carbon, ca., 1% of the bone specific activity at one week). The agreement with previously published data is very good.

After injection of 100 μ c (a 280 millicurie man-equivalent) approximately one year was required for the radiation being received by the "active area" of the bone to drop to the maximum tolerated level for man (0.04 rep/day).

This should not be considered as evidence that C^{14} is a particularly hazardous isotope since it is difficult to visualize accidental inhalation of millicurie quantities of C^{14}O_2 in any ordinary laboratory operation. These data would seem to suggest that the present allowable levels of C^{14} for specially authorized human experimentation (in the case of compounds in which the biological fate of the carbon isotope is C^{14}O_2) are not excessive.

SUMMARY

1. Studies of the retention of C^{14} from C^{14} -bicarbonate in the bones of mice have been carried over a period of 23 months.
2. Following injection of 100 μ c/25 gram mouse (a 280 mc man-equivalent) the radiation level in the most active portion of the bones remains above the maximum tolerated level for man (0.04 rep/day) for approximately one year.

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Table I

Comparison of Specific Activities of Long Bones from Mice Injected with $\text{NaHC}^{14}\text{O}_3$ Over Extended Periods

<u>Period After Injection</u>	<u>New Data</u>		<u>Skipper, Nolan and Simpson JBC 189, 189, 1951</u>	
	<u>Bone</u>	<u>Specific Activity (a)</u>	<u>Bone</u>	<u>Specific Activity (b)</u>
1 wk	Femur	1.5	Femur	1.8
	Humerus	1.2		
2 wk	Femur	1.5	Femur	1.5
1 mo	Femur	1.0	Femur	0.6
	Humerus	0.8		
4 mo	Femur	0.5	Femur	0.3
	Humerus	0.6		
6 mo			Femur	0.6
			Femur	0.5
8 mo	Femur	0.5	Femur	0.4
	Humerus	0.3		
12 mo	Tibia	0.16	Femur	0.15
	Humerus	0.15		
17 mo	Composite bone ^(c)	0.03		
22 mo	Composite bone ^(e)	0.02		

(a) Specific activity in $\mu\text{c}/\text{mole}$ of carbon.

(b) Specific activity in $\mu\text{c}/\text{mole}$ of carbon, corrected for comparison with new data by multiplying by 5.5 (18 μc of $\text{NaHC}^{14}\text{O}_3$ injected whereas 100 μc was used in the new experiments).

(c) Since the activities of bone after one year approached the limit of statistically significant counting, several long bones were used to provide larger samples for C^{14} assay.

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Table II

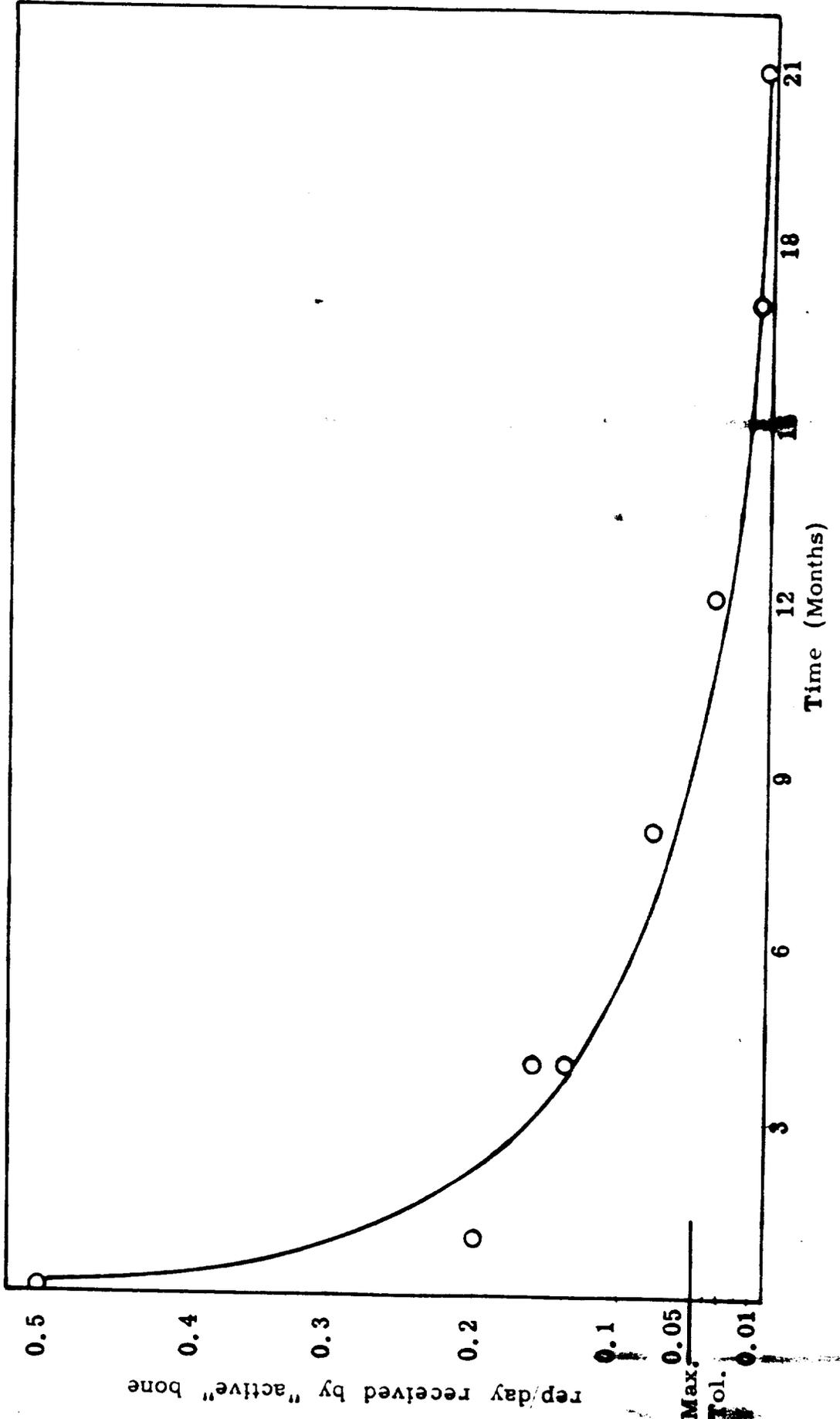
Calculations on Radiation Received per Day by "Active" Volume of Bone at Periods After Injection of $\text{Na}^{24}\text{CO}_3$ (100 μg)

<u>Period</u>	<u>Bone</u>	<u>Radiation Received by "Active" Bone (rep/day)</u>
1 wk	Femur	0.0
	Humerus	0.4
2 wk	Femur	<u>0.2</u>
1 mo	Femur	0.2
	Humerus	0.2
4 mo	Femur	0.14
	Humerus	0.17
6 mo	Femur	0.2
	Femur	<u>0.2</u>
	Femur	<u>0.17</u>
8 mo	Humerus	0.08
12 mo	Humerus	0.03
17 mo	Tibia and humerus	0.008
22 mo	Composite bone	0.005

Note: Underlined values taken from earlier data (JNC 150, 150, 1951) and corrected for difference in total C^{24} injected by multiplying by 5.5.

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Figure 1. Calculated radiation received by the area of bone where C^{14} from $NaHC^{14}O_3$ has localized at varying periods following single injection of 100 μc (a 230 mc man-equivalent).

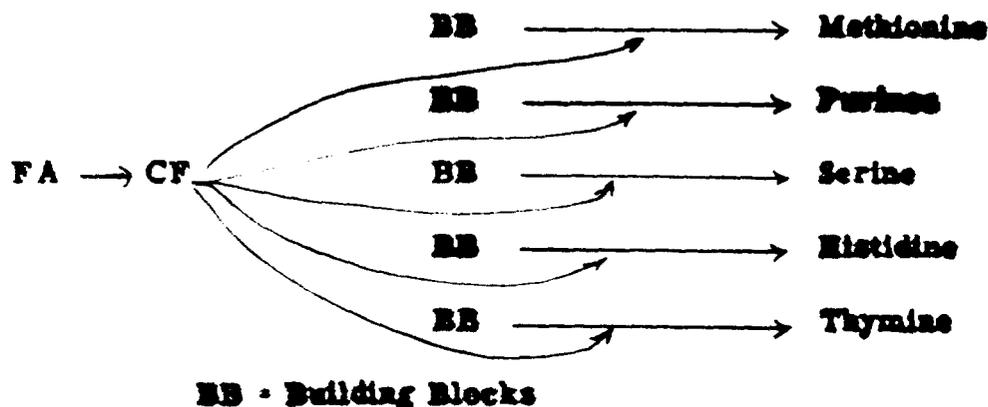


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**TRACER STUDIES WITH C¹⁴-LABELED FOLIC ACID IN MICE
BEARING A-METHOPTERIN SUSCEPTIBLE AND RESISTANT
STRAINS OF L1210 LEUKEMIA**

The present evidence suggests that folic acid (FA) is the precursor of tetrahydroformylpteroylglutamic acid (citrovorum factor), a coenzyme having to do with single-carbon unit transfers, notably those involving formate.

Reactions possibly involved may be depicted as follows:



A-Methopterin (4-amino-N¹⁰-methylpteroylglutamic acid) is an antagonist for folic acid or citrovorum factor and has been shown to inhibit conversion of folic acid to citrovorum factor (1).

Law has developed by treated transplantation a strain of L1210 leukemia which is dependent on A-methopterin for optimal growth (2). The original strain of L1210 leukemia is susceptible to A-methopterin as has been repeatedly demonstrated by life-span and local leukemia tumor mass growth experiments.

It has been reported that A-methopterin profoundly inhibits "de novo" nucleic acid synthesis (as measured by C¹⁴-formate incorporation) in L1210 A-methopterin sensitive leukemia (L1210-S) but more than doubles the rate of nucleic acid synthesis of the L1210-A-methopterin dependent (L1210-D) strain (2).

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Several possible mechanisms have been suggested to explain A-methopterin resistance in leukemic cells:

- (1) The transformed leukemic cells have acquired the ability to convert PGA antagonists into PGA or citrovorum factor.
- (2) The variant cells have acquired the ability to convert A-methopterin to a compound which can be utilized in biochemical reaction in lieu of PGA.
- (3) The resistant variants have acquired the ability to synthesize their own PGA or CF or have exceptional ability to convert PGA to CF and thus produce sufficient CF to overcome cellular levels of A-methopterin.
- (4) The resistant cells employ alternative metabolic pathways in purine, thymine, and amino acid synthesis.
- (5) The resistant or dependent cells possess apoenzymes with altered geometry and tighter binding of the metabolite (PGA) and ability to reject the antagonist.
- (6) The drug dependent cells produce an excess of the metabolite or a derivative thereof which in itself becomes inhibitory, thus the antagonist reduces the enzymatic activity to a more normal level stimulating growth.

The practical importance of this problem stems from the fact that A-methopterin, the best available temporary anti-leukemic agent in man, finally fails, probably because of a chemical selection of drug-resistant leukemic cells. Knowledge of the biochemistry of the resistant mutant is required before logical attempts toward simultaneously destroying the sensitive and the resistant leukemic cells is possible.

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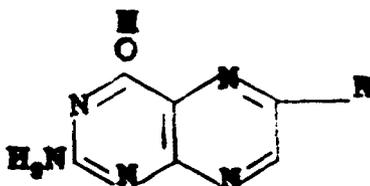
The present effort has been directed toward measuring the levels of C^{14} from folic acid-2- C^{14} in normal tissues and susceptible and dependent leukemic tumor masses in mice, some of which had been treated with A-methopterin before or after injection of C^{14} -folic acid.

EXPERIMENTAL

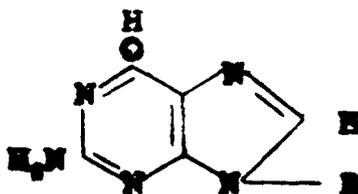
The folic acid-2- C^{14} used in this study was prepared by Weygand, Mann, and Simon (4) and was isolated in crystalline form by means of paper chromatography. This material had a specific activity of 1.7 $\mu\text{c}/\text{mg}$ and was injected into mice at levels of about 0.05 mg which contained approximately 0.05 to 0.1 μc per mouse. We are well aware of the fact that this is an unphysiologic dose of folic acid, but the specific activity of the labeled material and the limits of detection in tissue, dictated the injection level.

Mice (dba) were inoculated subcutaneously with leukemia L1210-S and L1210-D leukemias and after 6 or 7 days, some were treated with A-methopterin prior to injection of folic acid-2- C^{14} . After 6 or 24 hours the animals were sacrificed and certain tissues and the leukemic tumor masses were excised and oxidized to carbon dioxide which was precipitated as BaCO_3 prior to counting in a gas phase Geiger counter (5). Results obtained along with details of the experimental conditions are presented in Table I.

In view of the similarity of the pteridine nucleus of folic acid to the purine nucleus of nucleic acids:



Pteridine nucleus



Guanine

it was considered worth while to determine if the labeled atom of folic acid-2- C^{14} was incorporated into nucleic acids of mice to any considerable extent.

In these experiments C^{14} folic acid was injected into mice at a level of 0.1 μ c/day on three successive days and the mice were sacrificed at 6 hours after the last injection. The visceral nucleic acids were isolated as combined nucleic acids (CNA) and assayed for activity. The CNA specific activity in two such experiments was < 0.03 and < 0.03 μ c per mole of carbon respectively demonstrating that the pteridine nucleus of folic acid cannot be considered a precursor of nucleic acid purines.

DISCUSSION

If one assumed that the activity observed in the various animal tissues is due to free and bound folic acid and citrovorum factor (and not some degradation product of the 9-carbon atom of the labeled compound) the following statements could be made with regards to the present experiments:

1. The major portion of the labeled folic acid injected was rather rapidly excreted in the urine.
2. There is definite concentration of folic acid carbon in the liver (liver activity 14-37 times that of serum).
3. A-Methopterin profoundly inhibits incorporation of folic acid into the liver, small intestine, and leukemic tumors (either A-methopterin susceptible or dependent).
4. Folic acid incorporation into A-methopterin susceptible L1210 leukemic cells and A-methopterin dependent L1210 leukemic cells is of the same order.

The higher incorporation of C^{14} into the liver as compared to the intestine suggests that the labeled carbon atom of the C^{14} -folic acid is not excessively degraded to C^{14} -formate or $C^{14}O_2$, since extensive studies have shown that C^{14} from these two compounds is incorporated into the CNA of the intestine much more extensively than into the liver.

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The Effects of A-Methopterin on Tissue C¹⁴ Content Following Administration of Folic Acid-3-C¹⁴

Specific Activities (a)				
	L1210-S	L1210-S	L1210-A-Meth-D	L1210-A-Meth-D
Leukemia	L1210-S	L1210-S	L1210-A-Meth-D	L1210-A-Meth-D
Treatment	None	A-Meth	None	A-Meth
Period of Expt.	6 Hr	6 Hr	6 Hr	6 Hr
<u>Samples</u>				
Tumors 1	0.030	No Act.	0.043	0.019
2	0.032	No Act.	0.089	0.020
Liver	0.620	0.066	0.409	0.058
Sm. Intestine	0.063	0.026	0.080	< 0.01
Urine	71.3	60.0	62.0	5.12
Leukemia	L1210-S	L1210-S	L1210-A-Meth-D	L1210-A-Meth-D
Treatment	None	A-Meth	None	A-Meth
Period of Expt.	24 Hr	24 Hr	24 Hr	24 Hr
<u>Samples</u>				
Tumors 1	0.069	< 0.01	0.064	0.010
2	0.072	< 0.01	0.082	< 0.01
Liver	0.651	0.041	0.445	0.036
Sm. Intestine	0.206	0.021	0.143	0.013
Urine	11.0	26.1	2.17	4.73

Note: In the above experiments when A-methopterin was administered, it was given IP at 2.0 mg/kg on 1, 3, 5, and 7 days subsequent to leukemic inoculation and C¹⁴-folic was given IP immediately after the A-methopterin injection on the 7th day.

(a) Specific activities in $\mu\text{c}/\text{mole}$ of C.

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Leukemia	L1210-S	L1210-S	L1210-A-Meth-D	L1210-A-Meth-D
Treatment	None	A-Meth	None	A-Meth
Period of Expt.	24 Hr	24 Hr	24 Hr	24 Hr
<u>Samples</u>				
Tumors 1	0.103	0.056	0.048	0.040
2	0.196	0.036	0.136	0.037
Liver	1.11	0.473	0.246	0.376
Sm. Intestine	0.188	0.068	0.202	0.088
Serum	0.030	0.033	< 0.04	< 0.02
Adrenals	< 0.10	< 0.05	< 0.04	< 0.05
Kidneys	0.204	0.226	0.276	0.202

Note: In the above experiments when A-methopterin was administered, it was given on the 4th, 5th, and 6th days after leukemic transplantation and C^{14} -folic acid was given 1 hour before each A-methopterin injection (non-treated controls were given C^{14} -folic acid on the 4th, 5th, and 6th days). All experiments were terminated at 24 hours after the last C^{14} -folic acid injection.

SUMMARY

1. The major portion of the 3-labeled folic acid injected into mice is rather rapidly excreted in the urine.
2. There is definite concentration of the C^{14} from 3-labeled folic acid in the livers of mice.
3. A-Methopterin profoundly inhibits the C^{14} level (from C^{14} -labeled folic acid) in the livers, small intestines and leukemic tumors (either A-methopterin susceptible or A-methopterin dependent).
4. The C^{14} content of A-methopterin susceptible L1210 leukemic tumors and the A-methopterin L1210 dependent L1210 leukemic tumors is the same order at 6 and 24 hours after injection of 3-labeled folic acid.
5. Preliminary experiments suggest that the pteridine moiety of folic acid cannot be considered a precursor of nucleic acid purines.

REFERENCES

1. Nichol, C. A. and Welch, A. D. On the mechanism of action of aminopterin. Proc. Soc. Exper. Biol. and Med. 74, 403, 1951.
2. Law, L. W. and Boyle, P. J. Development of resistance to folic acid antagonists in a transplantable leukemia. Proc. Soc. Exper. Biol. and Med. 74, 599, 1950.
3. Skipper, H. E., Bennett, L. L., Jr. and Law, L. W. Effects of A-methopterin on formate incorporation into nucleic acids of susceptible and resistant leukemic cells. Cancer Res. 12, 677-679, 1952.
4. Weygand, F., Mann, H., and Simon, H. Synthese von Pteroyl-l-glutaminsäure-5-¹⁴C. Chemische Berichte 85, 463-465, 1952.
5. Skipper, H. E., Bryan, C. E., White, L., Jr., and Hutchison, O. S. Techniques for in vivo use of C¹⁴. J. Biol. Chem. 173, 371, 1948.

1111412

AUTORADIOGRAMS OF DROSOPHILA CHROMOSOMES

Brief mention has been made in past progress reports of the development of procedures which would allow for tracer studies on the chromosome level. The reasons for interest in chromosome incorporation of nucleic acid and amino acid precursors and the "in vivo" reaction of alkylating agents such as the mustards and ethylene imines with chromosomes have been pointed out.

Development of such techniques turned out to be more difficult than anticipated, but procedures have now been worked out which seem to be satisfactory. The procedure entails smashing of salivary gland chromosomes from *Drosophila* larva on a slide, immediately sealing the chromosomes under a cover glass with paraffin and later removing the cover glass by the dry-ice technique. The chromosome preparation is then covered with stripping film in the dark and placed in the cold for development of the autoradiogram. It is possible then to develop the stripping film without destroying the morphologic integrity of the chromosome or removing the stain.

Generally, the steps are as follows:

1. *Drosophila* are grown on banana agar.
2. When the larva have crawled up the side of the culture flask the salivary glands are dissected out in saline.
3. The glands are then transferred to 1 N HCl for approximately one minute.
4. The glands are then placed on a slide coated with a thin film of albumin and stained with aceto-orcein.
5. A cover slip is applied and the glands are smashed by pressing with the thumb and stroking with a blunt instrument.
6. The edges are temporarily sealed with a vaseline-paraffin.
7. The slides are then taken to a dark room where the cover slip is removed after freezing the slide on dry ice (flipped off with razor blade inserted under one edge of cover slip).
8. Stripping film is floated on water, allowed to expand and picked up with the slide, dried for about 10 minutes and placed in a dark

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box containing a dessicant. The box of stripping film coated slides is kept in the refrigerator during the development of the stripping film to prevent degradation of the chromosome and the development of latent image.

9. The film is developed in full strength D-19 for 5-10 minutes, washed in water, cleared with acid hypo, and washed for 20-25 minutes in gently flowing water. When the film is dried the excess film on the back of the slide is scraped off and permanent mount is made.

We have been through this procedure many times now and are sure that the morphology of the chromosomes is maintained and that the chromosomes do not cause "chemical-fogging" of the film.

Experiments are now under way using radioactive materials in the media on which larva are grown in first attempts at producing autoradiograms. It is probable that nucleic acid precursors incorporated into the cytoplasmic RNA will provide confusing background, but further refinement of the procedure might minimize this difficulty.

Submitted by

Howard E. Skipper
Howard E. Skipper

Martelia Bell
Martelia Bell

Linda Simpson
Linda Simpson

Birmingham, Alabama
April 24, 1953
15 6-121-KVII
ed (6)

Office Memorandum • UNITED STATES GOVERNMENT

TO : J. Wallace Ould, Jr., Assistant General Counsel DATE: April 29, 1952

FROM : R. G. Humphries, Acting Director, Contract Division

SUBJECT: REQUEST FOR RENEWAL OF CONTRACT AT-(40-1)-1038 - SOUTHERN RESEARCH INSTITUTE

SYMBOL: DC:RGH

Forwarded is an approved proposal for the extension of subject contract for another year beginning July 1, 1952. This approval action is covered by Procurement Directive ~~EM~~-52-204 dated April 10, 1952, in the amount of \$15,098.00. The contractor has reported that there will be no available balance for partial funding of the new period.

Accordingly, it is requested that you prepare an appropriate modification to continue this contract for another year on the basis outlined in the Contractor's proposal at a level of \$15,098.00.

Dr. C. S. Shoup will act as technical advisor on this contract action.



R.G. Humphries

Enclosures:

1. Memo fm Kasschau 4-17-52
2. Memo fm Tolbert 4-10-52
3. Contractor's proposal

CC: Dr. C. S. Shoup
Mr. A. A. Vergari
Mr. Ed. Ziegler
Mrs. J. Nicholson

/lm

1111415

Office Memorandum • UNITED STATES GOVERNMENT

TO John R. Moore, Director, Contract Division DATE April 17, 1952

FROM Kenneth Kasschau, Director of Research and Medicine

SUBJECT RENEWAL OF CONTRACT NO. AT-(40-1)-1038 - SOUTHERN RESEARCH INSTITUTE

SYMBOL: OR:AMC

We are enclosing a memorandum dated April 10, 1952, from the Division of Biology and Medicine, Washington, authorizing the renewal of the subject contract for one year, beginning July 1, 1952.

We would appreciate your preparing a modification to this contract. Dr. C. S. Shoup will act as technical advisor for this office.

K. Kasschau
Kenneth Kasschau

Enclosures:

1. Memo dtd 4-10-52 fm NET to KK
2. Ltr dtd 4-10-52 fm NET to HES
3. Procurement Directive
4. Status Rpt No. 13
5. Proposal

Corley:oc

*Approved by Procurement
Director BM-52-2 cd
4/10/52 — 15,078.00 ✓*

UNITED STATES ATOMIC ENERGY COMMISSION
DIVISION OF BIOLOGY AND MEDICINE
WASHINGTON, D. C.

DATE:

TO : Dr. Kenneth Hasselau, Director, Office
of Research & Medicine, Oak Ridge 00
FROM : H. E. Tolbert, Biochemist, Biology Br.
Division of Biology and Medicine
SUBJECT: TRANSMITTAL OF RESEARCH PROPOSAL FOR CONTRACT NEGOTIATION
SYMBOL : BMB:NET

This letter with enclosures, in triplicate, is sent in accordance with the procedure described in a letter from the General Manager to all Managers of Operations dated January 27, 1949.

1. Institution: Southern Research Institute
2. Investigator (s): Dr. Howard E. Skipper
3. Title: Body Retention of Carbon-14
4. () New Contract or (X) Renewal of Contract No. AT(40-1)-1038
5. Duration - From: July 1, 1952 To: June 30, 1953
6. AEC Technical Supervision: Division of Biology and Medicine
7. Recommended Support: \$15,098, includes 8% overhead
Authorized by Procurement Directive No. EM-52-204
Issued Apr 10 1952 \$ 15,098.00
Activity No. 6400
8. Other Comments:

This has been approved at \$15,098 rather than the amount requested, as there was an error in the calculation of the 8% overhead.

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8. Comments (Continued)

9. Security Requirements:

In accordance with the provisions of GM-93 (Revised March, 1950), and the requirements of the Declassification Guide, the Division of Biology and Medicine has determined that the following security precautions should be taken in connection with the proposed research contract.

Since there is essentially zero chance that restricted data may be required or developed, no personnel security requirements should be imposed.

- 10. Reports: (x) Reports are to be required as provided for by Memorandum Instruction of November 9, 1949, on subject "Direct Research Contract Reports".
- () Special Reports Instructions are as follows:

- Enclosures:
- (x) "A" - Proposal, dated _____
 - (x) "B" - Notification letter, dated _____
 - () "C" - Other correspondence, _____ letters
 - (x) "D" - Procurement Directive BR-52-204

Distribution:

Addressee: Original (w encl.)	Division File: Yellow Copy (w encl.)
1st Copy (" ")	Pink Copy (w/o encl.)
2nd Copy (" ")	Green Copy (" ")
Program Analysis	Branch File: White Copy (w ")
Branch: White Copy (w/o encl.)	

cc: Mr. Stanwood

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EMB:MET

APR 10 1952

**Dr. Howard E. Skipper
Assistant Director
Southern Research Institute
Birmingham 4, Alabama**

Dear Doctor Skipper:

We are glad to inform you that we have reviewed your request for renewal of your contract entitled, "Body Retention of Carbon-14," and that we are recommending to the Oak Ridge Operations Office that the contract be renewed for another year. We hope that you will be able to make continued progress on this project.

You may expect to hear from the Oak Ridge Operations Office within the near future regarding renewal of this contract.

Sincerely yours,

**N. Edward Tolbert
Biochemist, Biology Branch
Division of Biology and Medicine**

1111419

PROPOSAL
TO
ATOMIC ENERGY COMMISSION
DIVISION OF BIOLOGY AND MEDICINE
FOR
RENEWAL OF CONTRACT NO. AT-(40-1)-1038
ON
BODY RETENTION OF CARBON 14

SOUTHERN RESEARCH INSTITUTE

BIRMINGHAM, ALABAMA

FEBRUARY 21, 1952

1111420

BODY RETENTION OF CARBON 14

During the past year, data having to do with C¹⁴ retention and turnover have been or will be published shortly under the following titles:

1. Studies on the Hazard Involved in the Use of C¹⁴. III. Long-Term Retention in Bone. Skipper, Nolan, and Simpson, J. Biol. Chem. 189, 159, 1951.
2. Preferential Incorporation of Formate Carbon into Leukemic Blood Cells as Indicated by Autoradiography. Skipper, Chapman, Boyd, Riser, and Bell, Proc. Soc. Exper. Biol. Med. 77, 849, 1951.
3. Studies on the Hazard Involved in Use of Carbon 14. Howard E. Skipper, Nucleonics 10, 40, 1952.
4. Further Studies on Formate Incorporation by Leukemic Blood Cells. Skipper, Chapman, and Bell, Proc. Soc. Exper. Biol. Med. 78, 787, 1951.
5. The Failure of C¹⁴-Formate to Affect the Course of Mouse Leukemia. Skipper, Bell, and Chapman, Cancer Research, in press.

A reinvestigation of the long-term retention of C¹⁴ from bicarbonate carbon in bone and radiation calculations on dosages being received by the "active volume" of bones is under way. Excellent confirmation of previously reported results has been obtained up to one year after a single injection of 100 μc of $\text{NaHC}^{14}\text{O}_3$.

The effects of high levels of C¹⁴-formate (100-250 microcuries) on the development of spontaneous leukemia in Akm mice, on the life span of mice with Ak-4 leukemia, and on hematopoiesis in leukemic mice (Ak-4) have been

investigated. Results obtained suggest that these levels (280-750 millicurie man-equivalents) have no detectable effects under the conditions employed.

Extension of our studies on retention of C^{14} from various organic compounds has been possible. Data are now available on distribution and excretion of C^{14} from: methyl-labeled nitrogen mustard, ring-labeled triethylene melamine, 2,6-diaminopurine-2- C^{14} , 8-azaguanine-2- C^{14} , carbonyl-labeled urethan, methylene-labeled urethan, formate, and urea. Limited data are available on C^{14} -formyl folic acid and labeled guanidine.

Effort has been made to learn more about tissue localization of C^{14} from labeled formate and to develop techniques which will assist in studying localization of isotopic carbon on a cellular level.

PROPOSED PROGRAM

It is proposed that in our future efforts to gain information on the problem of C^{14} -hazard, the following lines of endeavor will be emphasized:

1. Continuation of the present investigation on long-term retention of bicarbonate carbon in bone and more particularly the localization in bone shaft and the radiation dosages received by areas of localization.
2. Extension of data on localization in areas of soft tissue having rapid mitotic and metabolic activity (tissue autoradiogram studies).
3. Attempt to extend knowledge of C^{14} -localization on a cellular level. Using giant cells of the salivary gland of *Drosophila*, it is planned to attempt microautoradiograms (following administration of C^{14} compounds) to determine localization in cell nuclei, chromosomes, nucleoli, and cytoplasmic

elements. See Figures 1 and 2 showing the smashed chromosomes of *Drosophila* (under oil) and the salivary gland cell nuclei showing chromosomes and nucleoli under lower magnification.

4. It is hoped that information on cellular distribution of C^{14} from radiomimetic compounds such as nitrogen mustard, triethylene melamine, and urethan will provide results of value from the standpoint of radiation biology. Consideration of the similarity of cytologic effects of the above agents and ionizing radiation will be taken into account in interpretation of data obtained.

BUDGET

A. Unexpended balance of contracted funds on January 31, 1952, \$5,222.82.

B. It is anticipated that these funds will be expended by the end of the contract period, July 1, 1952.

C. During the period of 1950-1951 while efforts were largely devoted to long-term bone work, it was possible to conserve \$6,979 of the contracted sum which was carried over with a request for a reduction of our 1951-1952 budget.

D. The work planned for the coming year (1952-1953) includes, in addition to continuation of prior efforts, developmental work on techniques for microautoradiography on a chromosome level. This will require a slightly larger budget than for the past year.

E. A detailed estimate of costs involved in carrying out the proposed research program is outlined below:

One Year (July 1, 1952-June 30, 1953)

(1) Salaries

Biochemist (part time)	\$ 4,500
Biologist (full time)	3,000
Biologist (full time)	3,200
Physicist (part time)	2,000
(2) Contribution to employees' retirement fund	430
(3) Radioactive compounds	300
(4) Glassware and animals	400
(5) Travel	150
(6) Overhead (8%)	1,184
	<hr/>
	\$15,164

Handwritten notes:
13 45
1 11
\$15,095

The contractor will furnish as its contribution to the project:

(1) Use of laboratory work space and equipment and facilities on hand.

(2) Clerical and administrative expenses and other general and administrative type costs in excess of the overhead allowance (8%).



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STATUS REPORT NO. 13
ON
BODY RETENTION OF CARBON 14
TO
ATOMIC ENERGY COMMISSION
DIVISION OF BIOLOGY AND MEDICINE
CONTRACT NO. AT-(40-1)-1038

Project No. 121

SOUTHERN RESEARCH INSTITUTE
BIRMINGHAM, ALABAMA

FEBRUARY 21, 1952

1214-121-XIII

1111427

BODY RETENTION OF CARBON 14

It has been pointed out in previous progress reports that with the information now available on retention of C^{14} from inhaled $C^{14}O_2$ (Brues and Buchanan) and aerosols of $BaC^{14}O_3$ (Southern Research Institute and California) and the long-term turnover studies of bicarbonate carbon in soft tissues and bone (SRI), it now becomes important to obtain more detailed information on areas of localization and slow turnover. This information should be gathered in such a way as to make possible radiation calculations regarding dosages received by areas of body tissues, particularly in the bone and possibly also in cellular areas known to be affected by ionizing radiation. It is also important to learn more about the carcinogenic action of C^{14} , if any, particularly at levels allowed in human experimentation and ranging downward to levels unavoidably inhaled or imbibed by laboratory workers employing this useful isotope. Finally, it seems important to learn more about the retention of C^{14} from a large spectrum of organic compounds in view of the increasing desire of clinicians to use C^{14} -labeled compounds in humans.

Our efforts during the past nine months have been directed toward gaining information on the above-mentioned problems.

Long-Term Retention of C¹⁴ in Bone
(and Localization in Bone Areas)

In an initial series of studies, NaHC¹⁴O₃ was injected into adult mice at a level of 18 microcuries (μc) and bone specific activities and total activities were determined over periods up to 13 months (J. Biol. Chem. 189, 159, 1951). Autoradiograms of long bones from these mice indicated that after a few weeks the remaining C¹⁴ was localized in the shaft (probably because of a slower turnover of carbonate carbon). Using these autoradiograms and knowledge of the total activity retained in corresponding long bones, it was possible to estimate the radiation (in r. e. p. per day) received by the bone cells in the "active area." It appeared that the "active area" of the bone shaft of mice injected with 18 μc NaHC¹⁴O₃ (a 50 mc man-equivalent) was receiving about 0.04 r. e. p. /day at five to six months after the single injection.

In view of the possible importance of such estimates to the problem of C¹⁴-hazard, it was decided to repeat these experiments using higher levels of C¹⁴-bicarbonate. A group of adult CFW strain mice was injected with 100 μc each of C¹⁴-bicarbonate and have been sacrificed as indicated in Table I. A femur and humerus were taken from each animal for oxidation and activity assay in a gas-phase counting apparatus which has been calibrated against a Bureau of Standards BaC¹⁴O₃ standard and shown to give results extremely close to the absolute C¹⁴ content. Corresponding bones have been fixed in alcohol, embedded in plastic, ground down to provide the desired section, and autoradiographed on No-Screen x-ray film. Results obtained to date are recorded in Table I. Previously reported data are included (with the necessary correction because of different doses) for comparison.

Table I
Calculations on Radiation Received by "Active" Bone
at Periods after a Single Injection of C¹⁴-Bicarbonate (100 µc)

<u>Period</u>	<u>Bone</u>	<u>Specific Activity</u> (µc/mole C)	<u>Average Radiation in</u> <u>"Active" Bone (r. e. p./day)</u>	
			<u>New</u> <u>Data</u>	<u>J. B. C. 189,¹</u> <u>159, 1951</u>
1 week	Femur	1.47	0.6	
	Humerus	1.17	0.4	
2 weeks	Femur	1.5 ¹		0.88
1 month	Femur	0.97	0.2	
	Humerus	0.82	0.2	
4 months	Femur	0.54	0.14	
	Humerus	0.54	0.17	
6 months	Femur	0.55 ¹		0.21
6 months	Femur	0.49 ¹		0.20
6 months	Femur	0.44 ¹		0.17
8 months	Femur	0.50		
	Humerus	0.30	0.08	
12 months	Tibia	0.18		on x-ray film
	Humerus	0.13		
12 months	Femur	0.15 ¹		

¹ These old values were corrected (x 5.5) because of the differences in C¹⁴ injected (18 vs. 100 µc) for comparison with the present results.

In addition, mice are still available which were injected with 100 μ c each of $\text{NaHC}^{14}\text{O}_3$ about 16 months ago. It is planned to sacrifice these mice at intervals of about four to six months. It is hoped that some will live greater than two years. These animals will be used to extend the above data.

We are happy to be able to report the very good agreement of our second series of experiments on turnover of C^{14} (in the "active area" of bone) with earlier experiments. When one considers the steps involved in these rough calculations which are based on carbon turnover up to one year in single animal experiments, autoradiograms (active areas), and the more precise physical measurement of absolute C^{14} content, such good agreement was not to be anticipated.

It is planned to attempt bone sectioning using a pressure system for embedding and preparation of micro-autoradiograms on additional mice injected with $\text{NaHC}^{14}\text{O}_3$ and perhaps on some of the >16-month old mice.

Tissue Micro-Autoradiograms

In our last progress report results were presented on C^{14} -formate-injected mice which showed the rapid uptake by tissue areas of most active metabolic and mitotic activity (e. g., crypts of Lieberkühn in the small intestine and the basement membrane of the seminiferous tubules of the testes).

The Institute has purchased a freeze-drying apparatus to allow for continuation of these studies. This equipment has now been assembled and is ready for use.

Cellular Autoradiography

From the standpoint of C^{14} -hazard, it is possibly of interest to learn whether following injection of C^{14} -labeled compounds, there is localization of isotope in cellular sites thought to be of importance in genetic function (nuclei, chromosomes). It is most assuredly of interest from the theoretical point of view (and with relation to radiation biology) to learn whether certain radiomimetic compounds such as nitrogen mustard, triethylene melamine, and urethan are preferentially fixed in the chromosomes, nucleoli, or in cytoplasmic elements.

With these points in view, we have begun some studies on the distribution of C^{14} -nitrogen mustard in salivary gland cells of larvae from *Drosophila* and smashed giant chromosome preparations from these cells. As soon as the techniques have been worked out, the studies will be extended to C^{14} -triethylene melamine and C^{14} -urethan both of which are radiomimetic (cause chromosome breaks and are carcinogenic, etc.).

We are lead to believe that this is feasible by results obtained on blood smear autoradiograms (Proc. Soc. Exper. Biol. Med. 77, 849, 1951, Skipper, Chapman, Boyd, Riser, and Bell). In these studies with 10-15 micron lymphocytes, very good autoradiograms were prepared. In the salivary gland cells we have chromosomes of about 150 microns in length and several microns between chromophylic bands (when stretched). These cells have nucleoli that are 20-30 microns in diameter and nuclei of the order of 100 microns in diameter.

It has been stated that "the nucleus of the cell is the center for the formation of protein, and thus for the primary growth process. This formation takes place with the mediation of nucleic acids which are of ribodesose type in the reproduction of the genes in the chromosome apparatus and of ribose type in the formation of the large protein masses in the cytoplasm. ----proteins are collected in the nucleolus and make up its main mass. From the nucleolus, they wander toward the nuclear membrane where with the mediation of ribose nucleic acids there takes place the formation of cytoplasmic protein" (Caspersson and Santesson, 1942). It is thought that the radiomimetic ethylene imines and nitrogen mustard because of their extremely active side chains combine with cellular proteins (nucleoproteins) and because these agents are bifunctional, there is cross-linking resulting in chromosome breaks.

If the discussed techniques can be made sensitive enough (and from the above this seems likely), useful information can almost certainly be obtained.

It is planned to determine whether any of a large number of C¹⁴-labeled compounds available in this laboratory can be observed to localize in cellular sites (i. e., C¹⁴O₂, C¹⁴-glycine, C¹⁴-urethan, C¹⁴-nitrogen mustard, C¹⁴-triethylene melamine, C¹⁴-8-azaguanine, C¹⁴-2, 6-diaminopurine).

These investigations seem to be a logical sequel to overall distribution and turnover studies in animals and tissue localization studies using autoradiography.

It is hoped that positive information on this subject will be available for our next progress report.

Formate Incorporation by Blood Cells

The details of a study on the incorporation of C¹⁴-formate into blood cells of leukemic mice are presented in the enclosed reprint (Further Studies on Formate Incorporation by Leukemic Blood Cells, Skipper, Chapman, and Bell, Proc. Soc. Exper. Biol. Med. 78, 786, 1951).

Formate Turnover in Mouse Tissues

Results of some rather extensive effort on the turnover of C¹⁴ from labeled formate in normal and neoplastic mice and rats are almost completed. This work carried out in cooperation with Dr. C. C. Stock of Sloan-Kettering Institute is in preparation for publication and will be included in the next progress report.

Submitted by Howard E. Skipper
Howard E. Skipper

Birmingham, Alabama
February 21, 1952
1214-121-XIII
mfw (20)

1111434

Further Studies on Formate Incorporation by Leukemic Blood Cells.* (19219)

HOWARD E. SKIPPER, JUANITA B. CHAPMAN, AND MARTELIA BELL.

From the Biochemistry Division, Southern Research Institute, Birmingham, Ala.

It has been observed that at one hour after injection of C^{14} -formate into mice with advanced Ak-4 leukemia, the greater portion of the leukemic prolymphocytes give positive autoradiograms on nuclear track emulsion while very few normal-appearing lymphocytes and practically none of the polymorphonuclears or erythrocytes are positive(1). Two preliminary explanations of these results were considered: (a) that the C^{14} -formate was incorporated during the anabolic phases of cell division and that the leukemic cells were dividing very rapidly as compared to normal cells, or (b) that the formed leukemic cells were metabolizing formate much more rapidly than were normal blood elements. The present study was designed to obtain information which might shed light on this question. In these experiments, leukemic blood has been exposed to C^{14} -formate away from sites of hematopoiesis for subsequent autoradiographic examination.

Experimental. Blood was aspirated from

* This work was supported by grants from the National Cancer Institute, of the National Institutes of Health, Public Health Service, and the Biology and Medicine Division of the Atomic Energy Commission.

the heart of Akm mice with advanced transplanted Ak-4 leukemia (a rather acute lymphoid strain). This blood was heparinized and placed in a small cellophane bag which was then introduced through an incision into the abdominal cavity of leukemic mice (Ak-4). These mice (with leukemic blood-containing cellophane bags) were injected IP. with 100 microcuries of C^{14} -formate. After one hour, the mice were sacrificed and blood smear autoradiograms were prepared on cardiac and cellophane-bag blood after each had been washed twice with inactive sera. The details of the procedure employed in making the autoradiograms have been presented(1). A number of blood smears on NTB plates (10 μ emulsion thickness) were made on each sample. The plates were developed for 2 or 20 minutes after exposure periods in the dark from 1 to 10 weeks. All of the NTB plates were examined under the microscope and a hundred or more cells on each were classified as grossly positive or negative. The results of these experiments are summarized in Table I.

Discussion. The white blood counts on the leukemic host animals in Exp. No. 1 and 2 were 7,500 and 34,800, respectively, somewhat

FORMATE INCORPORATION BY LEUKEMIC CELLS

TABLE I. Summary of Average C^{14} Formate Results on Cardiac and Cellophane Bag Blood

Exp. No.	Blood	Cardiac blood		Cellophane bag blood		Significance
		Function positive	% positive	Function positive	% positive	
1	Cardiac	16/428	3.7	98/192	51	0.198
	Cellophane bag	5/261	1.9	62/162	38	
2	Cardiac	12/240	2.9	67/120	56	0.214
	Cellophane bag	3/120	2.5	62/134	46	

In Exp. 1, the donor supplying the bag blood and the host animal were in the fifth leukemic day. Mice used in Exp. 2 were in the sixth leukemic day. Cells were counted in many randomly picked fields. All cells in each field were classified as positive or negative. Two observers counted each slide with rather good reproducibility.

lower than animals used in earlier experiments (1). The values of 51% and 56% positive large lymphocytes plus prolymphocytes (cells larger than 10μ in diameter) in the "host" mice are lower than the 90% positive prolymphocytes previously reported on cardiac blood in leukemic mice.

The per cent positive large lymphocytes and prolymphocytes in the cellophane bag blood was only slightly less than observed in cardiac blood from the "host" mice. This would seem to suggest that the profound difference between formate utilization in normal lymphocytes and leukemic prolymphocytes previously reported (at one hour after injection of C^{14} -formate) is a result of the more rapid uptake of the radioactive compound by formed leu-

kemic cells. It seems unlikely that blood cells immobilized in a cellophane bag in the abdominal cavity of a mouse are proliferating as rapidly as peripheral blood cell precursors; however, this possibility cannot be excluded by the present results.

Summary. Experiments have been carried out which demonstrate that leukemic cells immobilized in a cellophane bag implanted in the abdominal cavity of a host mouse incorporate C^{14} -formate much more rapidly than do normal lymphocytes.

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STATUS REPORT NO. 10
ON
BODY RETENTION OF CARBON 14
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PROJECT NO. 121

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BODY RETENTION OF CARBON 14

SUMMARY

1. Our initial studies on the long-term retention of C^{14} with particular attention to bone shaft have been summarized in a paper appearing in the Journal of Biological Chemistry 183, 153-166, 1951. Using higher levels of $NaHC^{14}O_3$, we are now in the process of carrying out experiments designed to provide information on the degree of radiation being received by areas in the bone over more extended periods. It is planned to employ microscopic autoradiograms to further refine the results obtained.

2. To date, we have observed no indication that 250 μc of C^{14} -formate (a 700 millicurie man-equivalent) given to leukemia susceptible mice affects the pattern of onset of spontaneous leukemia. Formate is a well-known precursor of carbon atoms in nucleic acid moieties.

3. Extensive studies on the distribution of formate carbon in normal and neoplastic mice are in progress. The status of this effort is described.

4. The preferential incorporation of formate carbon into leukemic blood cells as indicated by autoradiography is described in an enclosed manuscript.

5. The failure of C^{14} -formate (at levels of 100-200 μc per mouse) to affect the course of transplanted leukemia or to inhibit leukemic hematopoiesis is described.

BODY RETENTION OF CARBON 14

I. Long-Term Retention of C¹⁴ with Particular Attention to Bone Shaft

A paper entitled, "Studies on the hazard involved in use of C¹⁴.

III. Long-term retention in bone" by Skipper, Nolan, and Simpson has been published in the Journal of Biological Chemistry 189, 159-166, 1951. Reprints will be forwarded to the sponsor as soon as they become available.

As was mentioned in our last status report, additional experiments are under way in which mice have been given single doses of 100 microcuries (μc) of $\text{NaHC}^{14}\text{O}_3$ for the purpose of following long bone activities and carbon 14 distribution (by autoradiography). The specific activities of the femurs and the humeri of these mice at 24 hours, 1 week, 1 month, 4 months, 8 months, 12 months, 16 months, 20 months, 2 years, 2.3 years, 2.6 years, and 3 years (if we can persuade mice to live that long) will be determined. From data on the total activity in these bones, the integrated bone C¹⁴ content over the period in question, calculations on active areas in bone (as determined by autoradiograms), it will be possible to determine the average daily bone radiation (in r. e. p.), as well as the average daily radiation in the most active areas of the bone.

These new long-term experiments are now in their seventh month and bone sections embedded in plastic (from the 24-hour to the 4-month animals) are being exposed on No-Screen X-ray film.

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II. Effects of High Levels of $\text{HC}^{14}\text{OONa}$ on the Pattern of Deaths from Spontaneous Leukemia in Akm Mice

As was mentioned in our last progress report, paired litter mates of the highly inbred Akm strain mice are being injected at monthly intervals with active and non-active formate to determine if carbon 14 from this compound will affect the pattern of spontaneous leukemia. The experimental group has now been injected with a total of 250 microcuries per animal (5 monthly injections of 50 μc). The mean age of death from spontaneous leukemia in our line of this strain is about 9 months. This experiment has been under way for about 8 months now. Results to date are summarized in Table I.

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Table I

The Pattern of Deaths from Leukemia
 (Akm mice injected with HC¹⁴OONa compared with
 litter mate controls injected with non-radioactive formate)

Controls				HC ¹⁴ OONa Injected			
Animal No.	Sex	Litter	Age at Leukemic Death (days)	Animal No.	Sex	Litter	Age at Leukemic Death (days)
C789	F	A	219	RA789	F	A	
C790	F	B	258	RA790	F	B	
C791	F	C	271	RA791	F	A	241
C792	F	C	275	RA792	F	C	249
C793	F	D		RA793	F	D	250
C794	M	A		RA794	M	A	
C795	M	B	246	RA795	M	B	
C796	M	A	251	RA796	M	C	Died fighting ¹
C797	M	D		RA797	M	D	Died fighting
C798	M	D		RA798	M	D	Died fighting
C799	F	D	261	RA799	M	D	
C800	F	D	225	RA800	M	E	Died fighting
C801	F	E		RA801	M	E	
C802	F	E		RA802	M	F	Died fighting
C803	F	F	278	RA803	M	F	

¹ Five animals were killed in the early phases of this experiment before isolation of male mice.

Table II

Summary

	<u>Controls</u>	<u>HC¹⁴OONa</u>
Mortality data	9/15	3/10
Average age at leukemic death (days)	254	247

DISCUSSION

Unfortunately, five of the C¹⁴-formate mice were killed in fights in the early phase of this experiment, but the remaining 15 controls and 10 C¹⁴-formate injected litter mates will allow for a statistically significant comparison. To date, we have observed no indication that 250 µc of C¹⁴-formate (a 700 millicurie man-equivalent) given to leukemia-susceptible mice in 50 µc increments (at 2.5, 3.5, 4.5, 5.5, 6.5 months of age) affects the pattern of onset of spontaneous leukemia.

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III. The Overall Distribution of Formate Carbon in Normal and Neoplastic Animals

In our Status Report No. 9, certain data were presented regarding the distribution of formate carbon in normal and leukemic mice. These data strongly suggest that we have observed successful competition by neoplastic cells for formate carbon. In extension of these observations, mice with various types of tumors have been injected with C^{14} -formate and C^{14} -2,6-diaminopurine and sacrificed for comparison of the specific activity of the small intestine (the most active normal tissue) with the tumor. These studies are being carried out in cooperation with Dr. C. Chester Stock of the Sloan-Kettering Institute for Cancer Research. Table I shows experiments carried out to date in this comparison. Activity data are not yet available.

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Table I

Formate Distribution Studies

<u>Animal No.</u>	<u>Compound</u>	<u>Dose</u> <u>(μc/mouse)</u>	<u>Type Tumor</u>
1066-1067	2, 6-Diaminopurine	10	Sarcoma 180
1068-1069	HC ¹⁴ OONa	2	Sarcoma 180
1070-1071	2, 6-Diaminopurine	10	EO771
1072	HC ¹⁴ OONa	2	EO771
1077-1078	2, 6-Diaminopurine	5	Bashford carcinoma 63
1079-1080	HC ¹⁴ OONa	1.4	Bashford carcinoma 63
1081	HC ¹⁴ OONa	2	Ridgway osteogenic sarcoma
1082-1083	2, 6-Diaminopurine	2	Ridgway osteogenic sarcoma
1084-1085	HC ¹⁴ OONa	2	Mecca lymphosarcoma
1086-1087	2, 6-Diaminopurine	2	Mecca lymphosarcoma
1090-1091	2, 6-Diaminopurine	2	Mouse carcinoma 1025
1092-1093	HC ¹⁴ OONa	2	Mouse carcinoma 1025
1094-1095	2, 6-Diaminopurine	2	Ehrlich mouse Ascites tumor (solid form)
1096-1097	HC ¹⁴ OONa	2	Ehrlich mouse Ascites tumor (solid form)
1098-1099	HC ¹⁴ OONa	2	Wagner osteogenic sarcoma
1100	2, 6-Diaminopurine	2	Wagner osteogenic sarcoma
1105-1106	HC ¹⁴ OONa	2	Lewis mouse sarcoma T241
1107-1108	2, 6-Diaminopurine	2	Lewis mouse sarcoma T241
1109-1110	HC ¹⁴ OONa	2	Patterson lymphosarcoma
1111	2, 6-Diaminopurine	2	Patterson lymphosarcoma

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IV. Blood Smear Autoradiography

The incorporation of formate carbon into leukemic blood cells as indicated by autoradiography has been studied intensively during the past few months. The present status of this work is indicated in the manuscript appended to this report.

Well over one hundred nuclear track plates have been exposed in these studies. The incorporation of formate in the blood elements of normal animals, as well as incorporation of C^{14} from 2,6-diaminopurine- $2C^{14}$ and methyl bis(2-chloroethylamine)-methyl- C^{14} in normal and leukemic cells, is under investigation.

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V. The Failure of C¹⁴-Formate to Affect the Course of Transplanted Leukemia

Using the blood smear autoradiogram technique, it has been shown that leukemic cells (Ak-4 strain in Akm strain mice) are much more avid in the incorporation of C¹⁴ from formate than normal leukocytes (1). At one hour after the injection of 100 microcuries (μc) of C¹⁴-formate, 90% of the leukemic cells were highly active as compared to about 5% of the more mature lymphoid elements. No active polymorphonuclear leukocytes or thrombocytes were observed at one hour. These observations suggested the possibility that high levels of C¹⁴-formate might provide anti-leukemic action. The fact that formate is a precursor of the 2- and 8-carbon atoms of nucleic acid purines and the methyl carbon of desoxyribose nucleic acid thymine (2) (chromosome components) and knowledge of the short range of carbon radiation were considered encouraging. Knowledge of low energy of carbon 14 disintegration made the possibility less attractive.

EXPERIMENTAL

Two types of experiments have been carried out to date to determine the possible chemotherapeutic activity of C¹⁴-formate against experimental leukemia. The isotopic formate has been injected into Akm mice at two days after inoculation with Ak-4 leukemia and the life span of the treated mice compared with that of untreated leukemic controls. This general procedure for screening candidate anti-leukemic agents has been described previously (3). In a second

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and perhaps more sensitive assay of the effect of C¹⁴-formate on leukemic cells, mice with advanced leukemia have been injected with isotopic formate and the action on hematopoiesis followed by total white blood counts (tail vein counts).

The levels of isotopic carbon injected have been of the order of 100-200 μ c per mouse (approximately a 280-560 millicurie man-equivalent).

The results obtained relative to the effect of C¹⁴-formate on the life span of mice with Ak-4 leukemia are presented in Table I. The effect of 100 μ c of this material on the leukocyte counts of leukemic mice are summarized in Table II.

Table I

Observations on the Effect of 200 microcuries of
Radioactive Formate on the Life Span of Mice with Ak-4 Leukemia

<u>Treatment</u>	<u>Dosage (mg/kg)</u>	<u>Days of Leukemic Death</u>							<u>Average Life Span (days)</u>
		<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>	<u>11</u>	<u>12</u>	
Experiment I									
Inactive formate	0.035			4	2	3	1		9.1
Radioactive formate	0.035 and 200 μ c				3	1	1		9.6
Experiment II									
Inactive formate	0.017			2	2		1		9.0
Radioactive formate	0.017 and 100 μ c			3	1	1			8.6

Table II

The Effect of C¹⁴-Formate on the
White Blood Count of Mice with Ak-4 Leukemia

<u>Controls</u>	<u>Total White Blood Count</u>	
	<u>Range</u>	<u>Average</u>
5th day	7,800-10,300	8,760
6th day	15,000-26,800	22,000
7th day	52,600-95,400	76,000
8th day	154,000-172,800	164,200
<u>C¹⁴-Formate</u> <u>injected</u>		
6th day ¹	17,800-46,000	32,000
7th day	54,000-124,000	83,680
8th day	208,000-348,000	278,000 ²

¹ All of the experimental group of mice (5) were injected with 100 µc each of C¹⁴-formate on the 6th day after leukemic inoculation.

² Only two mice alive on 8th day.

DISCUSSION

The data presented in Tables I and II indicate that C¹⁴ from sodium formate when injected into leukemic mice at the level of 100-200 µc per animal has no perceptible effect on the leukemic process. The carbon 14-injected leukemic mice lived approximately the same period as non-active formate-

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injected controls. Certainly C¹⁴-formate had no tendency to inhibit leukemic cell production, Table II. This assay of the effect of the isotopic carbon on the blood counts of leukemic mice is considered a sensitive test of the radiation effects of C¹⁴. Although a disappointment from the standpoint of cancer chemotherapy, these results suggest that carbon 14 in fairly high levels is not apt to have much effect on mammalian hematopoiesis.

SUMMARY

C¹⁴-Formate when injected at levels of 100-200 μ c per mouse (280-560 millicurie man-equivalent) had no perceptible effect of leukemic life span on hematopoiesis.

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Birmingham, Alabama
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**PREFERENTIAL INCORPORATION OF FORMATE CARBON
INTO LEUKEMIC BLOOD CELLS AS INDICATED BY AUTORADIOGRAPHY¹**

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Demonstration of the incorporation of C¹⁴ into individual blood cells was first accomplished by Boyd, et al. (1) who showed by means of autoradiograms that the alpha carbon atom of glycine is taken up most rapidly by rat lymphocytes. The polymorphonuclear leukocytes incorporated C¹⁴ from glycine less rapidly and the circulating erythrocytes were considerably less avid in the utilization of the glycine carbon. The most obvious explanation for this difference in C¹⁴ content of the different cell types at 25 hours after injection appears to be the difference in their rate of formation (synthesis employing the alpha carbon of glycine as a precursor) and disappearance from the peripheral blood.

In the light of these results, it appeared of interest to study hematopoiesis in leukemic animals using the autoradiographic technique. Since the hematopoietic cell types and the peripheral blood cell types are probably exposed to the same concentrations of a labeled compound, preferential incorporation by neoplastic cells suggests (1) a difference in the rate of synthesis (if the labeled compound or a labeled degradation product is a normal metabolite), (2) a difference in the chemical

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reactivity of the precursor cells or formed elements, or (3) metabolic differences between the formed normal or neoplastic cells.

As a first approach to the study of hematopoiesis in experimental leukemia utilizing the autoradiographic technique, C¹⁴-labeled sodium formate has been employed. Formate has been reported to be a precursor of the 2- and 8-carbon atoms of desoxyribose nucleic acid (DNA) and ribose nucleic acid (RNA) guanine and adenine and the 5-methyl carbon of DNA thymine (2). Formate carbon has also been reported to appear in the alpha carbon of serine and in glycogen (3), in the methyl group of methionine, and the methyl groups of choline (4).

EXPERIMENTAL

Transplanted Ak-4 leukemia in Akm strain mice has been used in the present experiments. In this leukemia the number of abnormal forms in the differential counts parallels the rise in the total leukocyte count (5, 6). "The immature cells characteristic of Ak-4 leukemia were unlike the lymphocytes of normal mice. The young forms most closely resembling prolymphocytes were the largest cells observed and were often two or three times the size of neutrophils. The shape of the prolymphocytes varied from round to oval. The cytoplasm ranged from grey-blue to a darker intense blue and was usually coarse, uneven, and vacuolated. The appearance of the chromatin varied, sometimes slightly clumped, more typically, very clumped and pynotic" (5). Burchenal (5) further observed in the advance stages of this disease an occasional elevation of mature polymorphonuclear cells. However, because of the complete lack of intermediate myeloid forms,

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staining reactions, and general morphology, the term prolymphocyte was believed most applicable to these leukemic cells.

In our first experiments, leukemic mice of about two to three months of age were injected with 4.0 microcuries (μc) each of $\text{HC}^{14}\text{OONa}$. These mice were in the sixth day of the disease (transplanted Ak-4 leukemia) and had total leukocyte counts of the order of 100,000. Blood was taken directly from the heart of injected mice at six hours and the packed cells were washed twice with inactive mouse plasma. This washing was necessary to remove active sera which would cause confusing background in the final autoradiogram. The cells were then diluted with inactive mouse plasma (5:1) prior to making smears in a darkroom. Smears were made directly on Eastman NTB plates, emulsion thickness 10 microns, air dried, and exposed for six weeks (1). After exposure, the cells were fixed with methyl alcohol and the NTB plates were developed 2 or 20 minutes (the latter to bring out beta tracks) in full strength D-19. The plates were then cleared and the cells stained with Wright's stain.

In this first study, the autoradiograms which resulted from injection of 4.0 μc per mouse were faint even after six weeks' exposure. These autoradiograms were examined by an arbitrary technique which entailed counting silver grains immediately surrounding the individual cells. An eyepiece reticle which (under oil) divided a given field into 10 micron squares was placed in the microscope. Then, centering a given cell at the intersection of a four square area, the silver grains in the four squares ($20\ \mu \times 20\ \mu$) were counted while focusing up and down in order to observe grains in different planes. The background was counted in a number of fields where there were no cells and the average number of grains per

mm² determined. This average was subtracted from the values obtained around the cells examined giving a net count in the area immediately around the respective cells. These counts showed the prolymphocytes to have on an average about seven times as many silver grains (net particles per mm²) in the area immediately around the cell as did the small lymphocytes. The small lymphocytes had grain counts slightly above background. The polymorphonuclear cells and erythrocytes had no more silver grains in the surrounding area than average background.

In a second series of experiments, mice with advanced Ak-4 leukemia were injected with 100 microcuries each of C¹⁴-formate and blood smears made at one hour. After varying periods of exposure, the NTB plates were developed, stained, and examined. With this level of isotope, the autoradiograms were so pronounced after one week that it was relatively simple to classify cells as grossly positive or negative with regards to their radioactivity. NTB plates exposed for a period of greater than two weeks were so pronounced that the silver grains interfered somewhat with morphologic classification of cells. In view of this fact, it was decided to use cell diameter in addition to morphology in cell classification. The polymorphonuclear cells were easily classified since they were uniformly negative with regards to autoradiograms. Inactive leukemic blood smears on NTB plates carried through the development and staining procedure showed that normal lymphocytes ranged from about 6 to 9 microns in diameter. Prolymphocytes (leukemic cells) ranged in size from about 10-18 microns in diameter. There was a small percentage of cells in the lymphocytic series which were intermediate in size (10-12 μ) and which could not be unequivocally classified as normal large lymphocytes or leukemic cells. It was therefore considered most practical for

this initial work to report these autoradiograms on the following basis:

Polymorphonuclears	- identifiable by morphology
Lymphocytes	- 6-9 μ diameter
"Large" lymphocytes	- 10-12 μ diameter
Prolymphocytes	- 12-16 μ diameter

It is believed that on the basis of the morphology of these "large" lymphocytes, a goodly percentage may be leukemic cells. In any event, under this classification little opportunity prevails for mistaking the prolymphocytes (leukemic cells) and the normal lymphocytes.

Five observers have now studied a large number of autoradiograms of leukemic mouse blood at one hour after injection of 100 μ c of $\text{HC}^{14}\text{OONa}$. The agreement on the per cent of active cells of various types has been good. The results of these observations are given in Table I. Seven additional smears on NTB emulsion made from blood taken from mouse No. 2 were examined by two different observers. The additional results obtained were in good agreement with those reported in Table I. These autoradiograms were progressively darker with increased exposure and on 20-minute development in full strength D-19, beta tracks were in evidence. Typical autoradiograms are shown in Plates 1-2.

The possibility of chemical fogging in these studies has received considerable attention. In no instance have we observed chemical fogging in control smears from normal or leukemic mice. To date 65 control smears have been made on blood from 18 leukemic mice and 49 control smears have been made on blood from 11 normal mice. A fairly large number of normal and leukemic bone marrow smears from mice have also been prepared with no indication of chemical fogging.

DISCUSSION

From the results obtained, it appears that at one hour after injection of C^{14} -formate into mice with Ak-4 leukemia most of the prolymphocytes (leukemic cells) contain considerable amounts of the active atom, while the more mature cells are relatively inactive. A small percentage of the cells classed as normal lymphocytes were active though generally much less so than the prolymphocytes. In no instance in these one-hour experiments have we seen positive polymorpho-nuclear leukocytes, erythrocytes, or thrombocytes. Results obtained on peripheral blood and bone marrow have been parallel. These observations could be explained on the basis of the very rapid mitotic division and accompanying synthetic activity which must be existent in the leukemic hematopoietic tissue. Such an assumption would require the accompanying conclusion that there has been an almost complete turnover of leukemic cells within the period of these experiments while very few normal lymphocytes, polymorphs, or erythrocytes have been produced. A second and perhaps more reasonable conclusion which might be drawn from these data is the existence of a profoundly different rate of formate metabolism in formed leukemic and normal cells.

Any attempt to explain the present data on a biochemical level must take into consideration the level of citrovorum factor co-enzyme (thought to be involved in formate transfer) in normal and leukemic cells. Bethell (7) has observed that leukemic cells are considerably higher in citrovorum factor than are normal white blood cells. In view of the fact that:

(a) folic acid, which is converted to citrovorum factor (8),

will speed up the leukemic process (9),

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- (b) aminopterin inhibits formation of citrovorum factor (10) and incorporation of formate into nucleic acids (11),
- (c) folic acid inhibitors preferentially affect production of leukemic cells (5, 6) and increase the life span of leukemic mice (11, 12),
- (d) the anti-leukemic action of folic acid antagonists may be reversed by folic acid or citrovorum factor (14, 15), partially reversed by DNA or B₁₂ (16, 17), but is not reversed by formate, glycine, ascorbic acid, or choline (18),

several possibilities pertinent to the present discussion would seem to exist. It would appear that formate transfer via the above-mentioned co-enzyme may be a limiting factor in growth and cell division. Certainly the present data indicate that formate incorporation in leukemic cells (reportedly high in citrovorum factor) is much more active than in normal cells. It might be postulated, therefore, that leukemic cells geared to more active biosynthesis involving formate reflect more readily any inhibition of this limiting co-enzyme.

SUMMARY

At one hour after injection of 100 μ c of C¹⁴-formate into mice with advanced Ak-4 leukemia, it has been shown by means of blood smear autoradiograms that about 90% of the leukemic cells are highly active as compared to about 5% of the more mature lymphoid elements. No active polymorphonuclears, erythrocytes, or thrombocytes were observed within one hour.

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15. Burchenal, J. H., Babcock, G. M., Broquist, H. P., and Jukes, T. H. Prevention of the chemotherapeutic effects of 4-amino-N¹⁰-methylpteroylglutamic acid on mouse leukemia by citrovorum factor. *Proc. Soc. Exper. Biol. Med.* 71, 381, 1949.
16. Skipper, H. E., Chapman, J. B., and Bell, M. Partial reversal of the anti-leukemic action of folic acid antagonists by vitamin B₁₂. *Cancer Research* 11,

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161, 1951.

17. Skipper, H. E., Bell, M., and Chapman, J. B. Partial reversal of the anti-leukemic action of folic acid antagonists by nucleic acids. *Cancer*, in press.
18. Skipper, H. E., Bell, M., and Chapman, J. B. Unpublished results.

Table I

Per Cent Positive Blood Cell Autoradiograms Observed in Mice with

Advanced Ak-4 Leukemia at One Hour after Injection of 100 μ c of C¹⁴-Formate

Mouse No.	Observer	Origin	Total WBC	Differential Count				Per Cent Positive Autoradiograms			
				Polys	Prolymphs	Lymphs	"Large" Lymphs	Polys	Prolymphs	Lymphs	"Large" Lymphs
2	1	Blood	45,000	20	18	11	51	0	89	36	2
	2		14	13	9	53 ^a	0	100	50	5 ^a	
	3		18	20	10	52	0	94	50	1	
	4		19	21	10	52	0	90	35	2	
	5		--	--	--	--	0	82	78	6	
3	1 and 4	Marrow	47	12	11	30	0	87	58	7	
	1 and 4		--	--	--	--	0	94	67	13	
3	1 and 4	Blood	16.5	19	14	78	0	82	78	6	
	1		Marrow	--	--	--	--	0	86	55	9

Note: All observations are on random fields and include counts of 100 cells or more. The classification "'large' lymphs" includes lymphoid cells of 10-12 μ (difficult to classify morphologically because of the presence of silver grains in the nuclear track emulsion). A large percentage of these cells are believed to be leukemic.

a. This value included 10 "unidentified" negative cells in the range of 6-9 microns diameter.

Figure 1

Typical autoradiograms of prolymphocytes taken from leukemic mice one hour after injection of 100 μ c of C¹⁴-formate. In this case, the NTB plate was exposed in the dark for 3 weeks and developed for 20 minutes in full strength D-19. The cells were stained with Wright's stain.



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Figure 2

A positive prolymphocyte and a negative lymphocyte taken from a leukemic mouse one hour after injection of 100 μ c of C¹⁴-formate. In this example, the NTB plate was exposed for 2 weeks and developed for 2 minutes in full strength D-19.

1111464



1111465

Office Memorandum • UNITED STATES GOVERNMENT

TO : J. Wallace Ould, Jr., Assistant General Counsel DATE: May 24, 1951

FROM : R. G. Humphries, Contract Coordinator

SUBJECT: RENEWAL OF CONTRACT AT-(40-1)-1038-- SOUTHERN RESEARCH INSTITUTE

SYMBOL: CO:RGH

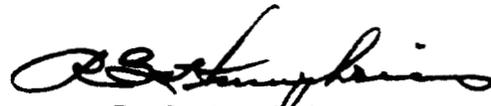
The Washington Division of Biology and Medicine has approved an extension of subject contract for another year with a budget of approximately \$11,000 covered by Procurement Directive BM-51-192.

The Contractor in submitting his proposal for extension of this contract did not furnish information concerning his expenditures during the course of the first year nor did he furnish a breakdown of the proposed budget for the second year.

This information has now been secured from the Contractor in his letter dated May 24, 1951, enclosed. It will be noted that the new budget has been detailed to cover a total amount of \$10,990. The present contract for the first year is in the amount of \$17,979, and the Contractor, in the same letter, has reported that he will expend by June 30, 1951, only \$11,000 of this amount which will leave a balance of \$6,979 available for partially funding the second year budget of \$10,990. Since this contract is on a lump sum basis this will mean that an additional lump sum payment of \$4,011 will be required to properly cover the two year contract amount of \$21,990.

Accordingly, it is requested that you prepare an appropriate modification to continue this contract for another year on a lump sum basis including the technical program outlined in the Contractor's proposal of April 13, 1951, and the breakdown of the budget as submitted in the letter dated May 24, 1951.

Dr. Edward McCrady will act as technical advisor on this contract action.


R. G. Humphries

Enclosures:

Memo fm Woodruff 5-18-51 w/
Ltr. fm Contractor 5-24-51
Memo fm Pearson 5-4-51 and
Contractor's proposal

CC: Dr. Edward McCrady
Mr. Ed. Ziegler

Humphries:lm

1111466

SOUTHERN RESEARCH INSTITUTE

917 SOUTH TWENTIETH STREET
BIRMINGHAM 5, ALABAMA



May 24, 1951

Mr. Glenn Humphries
Atomic Energy Commission
P. O. Box E
Oak Ridge, Tennessee

Dear Mr. Humphries:

In reply to the points raised in our telephone conversation of May 21st, the following information is listed with regards to renewal of our Contract No. 121, "Body Retention of C¹⁴":

1. As of April 30, 1951, we had expended \$8,400.86 of the contract amount.

2. We expect to have spent about \$11,000 by June 30, 1951, the expiration date of the contract.

3. The breakdown of the \$11,000 budget for the coming year (July 1, 1951-June 30, 1952) is submitted as follows:

Salaries (including sick leave, vacation, and holiday pay):		
Biochemist (part time)	\$4,000	
Biologist (full time)	2,800	
Physicist (part time)	1,875	
Physical chemist (part time)	500	
Total salaries		\$ 9,175
Contributions to employees' regular retirement fund		321
Apparatus and equipment - nothing required		
Expendable supplies:		
Radioactive compounds		300
Glassware and animals		230
Travel		150
	Subtotal	<u>\$10,176</u>
Overhead at 8% of above costs		814
	Total	<u>\$10,990</u>

1111468

Mr. Glenn Humphries
Atomic Energy Commission
Page 2
May 24, 1951

If any further information is required, please do not hesitate to
call on us.

Yours very truly,

Howard E. Skipper
Howard E. Skipper
Assistant Director

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1111469

UNITED STATES ATOMIC ENERGY COMMISSION
DIVISION OF BIOLOGY AND MEDICINE
WASHINGTON, D. C.

DATE: MAY 4 1951

TO : Nathan Woodruff, Director, Research and
Medical Division, Oak Ridge Operations Office
FROM : Paul B. Pearson, Chief, Biology Branch *PBP*
Division of Biology and Medicine
SUBJECT: TRANSMITTAL OF RESEARCH PROPOSAL FOR CONTRACT NEGOTIATION
SYMBOL : BMB:PBP

This letter with enclosures, in triplicate, is sent in accordance with the procedure described in a letter from the General Manager to all Managers of Operations dated January 27, 1949.

1. Institution: Southern Research Institute
2. Investigator (s): Dr. Howard E. Skipper
3. Title: "Body Retention of Carbon 14."
4. () New Contract or (x) Renewal of Contract No. AT(40-1)1038
5. Duration - From: July 1, 1951 To: June 30, 1952
6. AEC Technical Supervision: Division of Biology and Medicine
7. Recommended Support: \$11,000

Authorized by Procurement Directive No. EM-51-192

Issued MAY 4 1951 \$ 11,000

Activity No. 6400

8. Other Comments: The budget approved is in accordance with conversation with Dr. Skipper since he submitted his report and request for renewal. It is felt that 10 or \$11,000 will be adequate to cover the work planned on the project for the ensuing year.

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1951
A-3150

8. Comments (Continued)

9. Security Requirements:

In accordance with the provisions of GM-93 (Revised March, 1950), and the requirements of the Declassification Guide, the Division of Biology and Medicine has determined that the following security precautions should be taken in connection with the proposed research contract.

Since there is essentially zero chance that restricted data may be required or developed, no personnel security requirements should be imposed.

10. Reports: (x) Reports are to be required as provided for by Memorandum Instruction of November 9, 1949, on subject "Direct Research Contract Reports".
- () Special Reports Instructions are as follows:

- Enclosures: (x) "A" - Proposal, dated _____
- (x) "B" - Notification letter, dated MAY 1 1951
- () "C" - Other correspondence, _____ letters
- (x) "D" - Procurement Directive EM-51-192

Distribution:

Addressee: Original (w encl.)	Division File: Yellow Copy (w encl.)
1st Copy (" ")	Pink Copy (w/o encl.)
2nd Copy (" ")	Green Copy (" ")
Program Analysis	Branch File: White Copy (w ")
Branch: White Copy (w/o encl.)	

Dr. Howard E. Skipper
Assistant Director
Southern Research Institute
Birmingham, Alabama

Dear Doctor Skipper:

I am glad to advise you that we have reviewed your request for renewal of your contract entitled, "Body Retention of Garter 14," and that we are recommending to the Oak Ridge Operations Office that the contract be renewed for another year.

We hope that you will be able to make continued progress on this project as it is of definite interest to the Commission.

You may expect to hear from the Oak Ridge Operations Office within the near future regarding renewal of this contract.

Sincerely yours,

Paul E. Pearson
Chief, Biology Branch
Division of Biology and Medicine

UNITED STATES ATOMIC ENERGY COMMISSION
DIVISION OF BIOLOGY AND MEDICINE
WASHINGTON, D. C.

DATE: MAY 4 1951

TO : Nathan Woodruff, Director, Research and
Medical Division, Oak Ridge Operations Office
FROM : Paul B. Pearson, Chief, Biology Branch
Division of Biology and Medicine
SUBJECT: TRANSMITTAL OF RESEARCH PROPOSAL FOR CONTRACT NEGOTIATION
SYMBOL : BMB:PBP

This letter with enclosures, in triplicate, is sent in accordance with the procedure described in a letter from the General Manager to all Managers of Operations dated January 27, 1949.

1. Institution: Southern Research Institute
2. Investigator (s): Dr. Howard E. Skipper
3. Title: "Body Retention of Carbon 14."
4. () New Contract or () Renewal of Contract No. ~~49(40-2)1038~~
5. Duration - From: July 1, 1951 To: June 30, 1952
6. AEC Technical Supervision: Division of Biology and Medicine
7. Recommended Support: \$11,000

Authorized by Procurement Directive No. 51-192

Issued _____ \$ 11,000

Activity No. 6400

8. Other Comments: The budget approved is in accordance with conversation with Dr. Skipper since he submitted his report and request for renewal. It is felt that 10 or \$11,000 will be adequate to cover the work planned on the project for the ensuing year.

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8. Comments (Continued)

9. Security Requirements:

In accordance with the provisions of GM-93 (Revised March, 1950), and the requirements of the Declassification Guide, the Division of Biology and Medicine has determined that the following security precautions should be taken in connection with the proposed research contract.

Since there is essentially zero chance that restricted data may be required or developed, no personnel security requirements should be imposed.

10. Reports: (X) Reports are to be required as provided for by Memorandum Instruction of November 9, 1949, on subject "Direct Research Contract Reports".
- () Special Reports Instructions are as follows:

- Enclosures: (X) "A" - Proposal, dated _____
- (X) "B" - Notification letter, dated _____
- () "C" - Other correspondence, _____ letters
- (X) "D" - Procurement Directive BL-51-192

Distribution:

Addressee: Original (w encl.)	Division File: Yellow Copy (w encl.)
1st Copy (" ")	Pink Copy (w/o encl.)
2nd Copy (" ")	Green Copy (" ")
Program Analysis	Branch File: White Copy (w ")
Branch: White Copy (w/o encl.)	

Dr. Howard E. Skipper
Assistant Director
Southern Research Institute
Birmingham, Alabama

Dear Doctor Skipper:

I am glad to advise you that we have reviewed your request for renewal of your contract entitled, "Body Retention of Carbon 14," and that we are recommending to the Oak Ridge Operations Office that the contract be renewed for another year.

We hope that you will be able to make continued progress on this project as it is of definite interest to the Commission.

You may expect to hear from the Oak Ridge Operations Office within the near future regarding renewal of this contract.

Sincerely yours,

Paul A. Pearson
Chief, Biology Branch
Division of Biology and Medicine

UNITED STATES ATOMIC ENERGY COMMISSION
DIVISION OF BIOLOGY AND MEDICINE

WASHINGTON, D. C.

8. Comments (Continued)

DATE: MAY 4 1951

TO : Nathan Woodruff, Director, Research and
Medical Division, Oak Ridge Operations Office
FROM : Paul B. Pearson, Chief, Biology Branch
Division of Biology and Medicine
SUBJECT: TRANSMITTAL OF RESEARCH PROPOSAL FOR CONTRACT NEGOTIATION

SYMBOL : BMB:PEP

9. Security Requirements:

This letter with enclosures, in triplicate, is sent in accordance with the provisions of 48 CFR (Revised March 1950) and the requirements of the Security Regulations of the General Manager to all Managers of Operations under the Division of Biology and Medicine has determined that the following security precautions should be taken in connection with this information: ~~Western Research Institute~~

Since this information is classified as Restricted data, it may be furnished only to those personnel who have been granted access to Restricted data in accordance with Section 11.2

4. () New Contract of () Renewal of Contract No. ~~48(40-111000)~~

5. Duration - From: July 1, 1951 To: June 30, 1952
10 Reports: Reports are to be required as provided in 11.2

6. AEC Technical Supervision: Division of Biology and Medicine

7. Recommended Support: \$11,000

Authorized by Procurement Directive No. PL-51-192

Issued MAY 4 1951 \$ 11,000

Activity No. 6400

8. Other Comments: The budget approved is in accordance with negotiation with Dr. Skipper since he submitted his report and request for renewal. It is felt that 10 or \$11,000 will be adequate to cover the work planned on the project for the ensuing year.

Distribution:

Addresses: Original (w/ encl.)	Division file: 1st Copy (w/ encl.)
1st Copy (" ")	Pink Copy (w/o encl.)
2nd Copy (" ")	Green Copy (" ")
Program Analysis	Branch file: White Copy (w/ encl.)
Branch: White Copy (w/o encl.)	

MAY 1 1951

**Dr. Edward E. Skipper
Assistant Director
Southern Research Institute
Birmingham, Alabama**

Dear Doctor Skipper:

**I am glad to advise you that we have reviewed your request for
renewal of your contract entitled, "Boly Substitution of Carbon 14,"
and that we are recommending to the Oak Ridge Operations Office
that the contract be renewed for another year.**

**We hope that you will be able to make continued progress on this
project as it is of definite interest to the Commission.**

**You may expect to hear from the Oak Ridge Operations Office within
the near future regarding renewal of this contract.**

Sincerely yours,

**Paul E. Pearson
Chief, Biology Branch
Division of Biology and Medicine**

1111477

PROPOSAL
TO
ATOMIC ENERGY COMMISSION
DIVISION OF BIOLOGY AND MEDICINE
FOR CONTINUATION OF
A RESEARCH PROGRAM
ON
BODY RETENTION OF CARBON 14
PROJECT NO. 121

SOUTHERN RESEARCH INSTITUTE

BIRMINGHAM, ALABAMA

APRIL 13, 1951

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BODY RETENTION OF CARBON 14

a. Scope and Present Literature

The entrance of carbon from carbon dioxide into both organic and inorganic metabolic pathways has been established. This fact, coupled with knowledge of the half life of C^{14} (approximately 5,000 years), has led to considerable concern regarding hazard to persons utilizing this most important isotope. Information as to the degree and duration of tissue exposure following the intake of compounds labeled with C^{14} , as well as knowledge of the effect of such exposures on organs, tissues, and cells, is required if the hazard accompanying the use of this isotope is to be properly assessed. In an attempt to obtain information which will be useful in a final assessment of the hazard involved in use of C^{14} , ^{the Contractor has} ~~we have~~ employed the following as guiding principles:

- (1) The two most probable sources for inadvertent C^{14} intake by the laboratory worker are $C^{14}O_2$ and $BaC^{14}O_3$.
- (2) In view of (1) above, it becomes of greatest importance to determine the rate of turnover of C^{14} from $C^{14}O_2$ in organs, tissues, and areas in tissues (down to cellular dimensions, since C^{14} emits beta rays that traverse but several cell diameters).
- (3) It is imperative to learn more about the retention of C^{14} from inspired $BaC^{14}O_3$ under the most realistic conditions.
- (4) Since carcinogenesis is the greatest biologic danger from C^{14} ingestion, studies should be carried out to learn if

reasonable doses of this isotope affect the onset of neoplastic diseases such as leukemia.

~~Research to date has provided information which has been presented~~
in the following publications:

1. Studies on the hazard involved in use of C^{14} . I. Retention of carbon from labeled sodium bicarbonate. Skipper, White, and Bryan. J. Biol. Chem. 180, 1187-1195, 1949.
2. Studies on the hazard involved in use of C^{14} . II. The effect of a single dose of C^{14} -labeled sodium bicarbonate on the pattern of deaths from spontaneous leukemia in Akm mice. Skipper, Bell, and Chapman. Cancer Research 10, 362-363, 1950.
3. Studies on the hazard involved in use of C^{14} . III. Long-term retention in bone. Skipper, Nolan, and Simpson. J. Biol. Chem. 189, 159-167, 1951.

Other points having to do with carbon 14 turnover have been covered in:

4. Carbamates in the chemotherapy of leukemia. VIII. Over-all tracer studies on carbonyl-labeled urethan, methylene-labeled urethan, and methylene-labeled ethyl alcohol. Skipper, Bennett, Bryan, White, Newton, and Simpson. Cancer Research 11, 46, 1951.
5. Inhibition of nucleic acid synthesis by folic acid antagonists. Skipper, Mitchell, and Bennett. Cancer Research 10, 510, 1950.
6. Effect of x-radiation on the biosynthesis of nucleic acids and nucleic acid purines. Skipper and Mitchell, Cancer, in press.

Papers in preparation are listed below:

7. Preferential incorporation of formate carbon into leukemic blood cells as indicated by autoradiography. Skipper, Chapman, Boyd, Riser, Bell, and Burchenal.

8. Studies on the hazard involved in use of C¹⁴. IV. The fate of C¹⁴ inspired as a BaC¹⁴O₃ aerosol.

IP Research during the initial period of performance has added to the knowledge of body retention of C¹⁴. That research in summary, we have obtained information suggesting that long-term

retention of C¹⁴ in bone, and more specifically, certain areas of the bone, is of greatest importance to the ~~subject~~ problem. Longer experiments (> one year) including quantitative C¹⁴ determination, area distribution as measured by autoradiograms, and radiation calculations in bone are ~~considered~~ most important.

(As Contractor 445)
To date, ~~we have~~ obtained no information suggesting that fairly high levels of C¹⁴-bicarbonate or C¹⁴-formate hasten the onset of spontaneous leukemia in Akm mice. In fact, ^{the} ~~our~~ findings to date are encouraging from the standpoint of the lack of danger to users of this isotope.

EXPERIMENTAL

to be carried forward
b. Plan of Approach

~~It is proposed that~~ (1) the longer term experiments on C¹⁴ turnover in bone ^{will} be continued, (2) attempts ^{will} be made to obtain more information on C¹⁴ localization (and local radiation) in bone and soft tissues by use of microscopic autoradiograms, (3) continued efforts to observe biologic effects from high levels of C¹⁴ ^{will} be made, (4) work ^{will} be continued on the fundamental processes of

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hematopoiesis using the blood smear autoradiogram technique, and (5) further work be carried out toward determination of the rate constants of the reactions involved in turnover of C^{14} from $C^{14}O_2$ employing analog computers.

ESTIMATED COST OF PROGRAM (1 YEAR)

It is proposed that the program be continued for another year at the present rate of expenditure.

Birmingham, Alabama
April 13, 1951
mfw (7)

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Office Memorandum • UNITED STATES GOVERNMENT

TO : D. Vanden Bulck, Assistant to the Manager

FROM : Albert H. Holland, Jr., M.D., Director of Research and Medicine

SUBJECT: RESEARCH CONTRACT - SOUTHERN RESEARCH INSTITUTE

SYMBOL: RM:ALC

DATE: May 26, 1950

Lump Sum

We are transmitting a memorandum from Paul B. Pearson, Chief, Biology Branch, Division of Biology and Medicine, Washington, dated May 23, 1950, along with the enclosures, requesting a research contract be negotiated with the Southern Research Institute, Birmingham, Alabama. The title of the project will be "Body Retention of Carbon 14".

Dr. Harry Stoeckle, Medical Advisor, Office of Research and Medicine, will be the technical representative to contact for making arrangements for negotiation of the contract.

for *John Robinson*
 Albert H. Holland, Jr., M.D.

Encl.:
 memo with encls.

CC: Dr. Stoeckle

Corley:tw

R.G. Humphries

Allen

A memo is being sent out to me

regarding labeled compounds.

I wonder whether you would like to know

that information. Is it?

UNITED STATES ATOMIC ENERGY COMMISSION
DIVISION OF BIOLOGY AND MEDICINE
WASHINGTON, D. C.

TO : A. H. Holland, Jr. Chief, DATE: 23 May 1950
Medicine and Research Division, Oak Ridge
FROM : Paul B. Pearson, Chief, Biology Branch PBP
Division of Biology and Medicine
SUBJECT: TRANSMITTAL OF RESEARCH PROPOSAL FOR CONTRACT NEGOTIATION

REFER TO

SYMBOL: BMB:PEP

This letter with enclosures, in triplicate, is sent in accordance with the procedure described in a letter from the General Manager to all Managers of Operations dated January 27, 1949.

1. Institution: Southern Research Institute
Birmingham, Alabama
2. Investigator(s): Howard E. Skipper
3. Title: "Body Retention of Carbon 14"
4. Duration - From: July 1, 1950 To: 1 year from date of contract
5. AEC Technical Supervision: Division of Biology and Medicine
Biology Activity #6400
6. Recommended Support: \$17,979 (including 8% overhead)
7. Other Comments
This is a continuation of a project that the AEC has supported through the Office of Naval Research.

PROPOSAL FOR A
CONTINUATION OF
THE RESEARCH PROGRAM

ON
BODY RETENTION OF CARBON 14

TO
ATOMIC ENERGY COMMISSION
DIVISION OF BIOLOGY AND MEDICINE

1111486

SOUTHERN RESEARCH INSTITUTE

BIRMINGHAM, ALABAMA

MARCH 29, 1950

PROPOSAL NO. 184

BODY RETENTION OF CARBON 14

BACKGROUND

The program underway at Southern Research Institute on the hazard involved in use of carbon 14 has been concerned with:

(1) Quantitative data on the retention of this isotope in various organs and tissues (and the whole body) at extended periods following injection of $\text{NaHC}^{14}\text{O}_3$.

(2) Rate of turnover data on carbon 14 (from $\text{NaHC}^{14}\text{O}_3$) in the whole long bone versus the diaphyseal bone (shaft). These studies involve both dissection and quantitative activity assays and autoradiography.

(3) Investigation of the effect of a given dose (50 mc man-equivalent) of $\text{NaHC}^{14}\text{O}_3$ on the pattern of deaths from leukemia in Albn mice. Similar studies are under way with a C^{14} -labeled compound known to enter into chromosome metabolism, 2,6-diaminopurine.

(4) Study of the over-all distribution and rate of turnover of carbon 14 in certain labeled organic compounds of biological interest (carbonyl and ethoxy-labeled urethan, methylene-labeled ethyl alcohol, urea, 2-labeled 2,6-diaminopurine, and 2-labeled 8-azaguanine).

(5) Experiments having to do with the retention and turnover of $\text{BaC}^{14}\text{O}_3$ when breathed in as an aerosol of the particle size known to be retained in the human lung.

(6) Rate-constant studies on C^{14}O_2 fixation in the carcass and carcass fractions (fat, protein, and glycogen) of mice.

(7) Fixation of carbon 14 from $\text{NaHC}^{14}\text{O}_3$ and HC^{14}OOH in chromosomal fractions.

Extensive experimental and calculated data have been reported previously on the retention of carbon from labeled sodium bicarbonate. These data which included results up to three months following injection of $\text{NaHC}^{14}\text{O}_3$ were the subject of two recent publications: Studies on the Hazard Involved in Use of C^{14} . I. Retention of Carbon from Labeled Sodium Bicarbonate by Howard E. Skipper, Locke White, Jr., and Carl E. Bryan, *J. Biol. Chem.* 189, 1187, 1949, and Body Retention of Carbon 14 from Labeled Sodium Bicarbonate, *Science* 110, 306, 1949, by the same authors. Our further studies on this subject will soon be the subject of another paper to include retention data up to one year following injection of $\text{NaHC}^{14}\text{O}_3$. This publication will emphasize diaphyseal bone carbon 14 turnover versus whole bone carbon turnover.

Another phase of the program has now reached a stage where a formal report in the literature seems indicated. In accordance with the policies of the sponsors encouraging publication, we have prepared and submitted a manuscript entitled "Studies on the Hazard Involved in Use of Carbon 14. II. The Effect of a Single Dose of C^{14} -Labeled Sodium Bicarbonate on the Pattern of Deaths from Spontaneous Leukemia in Akm Mice" by Howard E. Skipper, Martelia J. Bell, and Juanita B. Chapman to Cancer Research. This paper will appear in an early issue.

An almost identical experiment is now under way in which Akm mice have been injected with a 5 mc man-equivalent of labeled 2,6-diaminopurine (known to be a precursor of nucleic acid guanine) and set aside to await death from spontaneous leukemia.

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Our studies on the over-all distribution and turnover of carbon 14 from labeled organic compounds has progressed to the point where publication of certain of the results seems indicated. A manuscript entitled "Carbamates in the Chemotherapy of Leukemia. VIII. Over-all Tracer Studies on Carbonyl-Labeled Urethan, Ethoxy-Labeled Urethan, and Methylene-Labeled Ethyl Alcohol" by Howard E. Skipper, Leonard L. Bennett, Jr., Carl E. Bryan, Margaret Ann Newton, and Linda Simpson has been prepared.

Experiments having to do with the over-all distribution and turnover of carbon 14 from labeled 2,6-diaminopurine and 8-azaguanine (guanazolo) have been carried out.

PROPOSED PROGRAM

In our studies to date we have attempted to emphasize investigation of the most immediate hazards to workers using carbon 14 (tissue and bone radiation following exposure to $C^{14}O_2$ or $BaC^{14}O_3$ aerosol) and for purposes of safety have used relatively low levels of activity and small animals (18 μ c/mouse or a man-equivalent of about 50 mc/man). With the generally encouraging data now at hand regarding safety of experimentation with carbon 14, it is suggested that much more stringent experiments should be carried out to determine what dosages of a seemingly more specific carbon 14-containing compound are required to produce radiation effects.

It is now planned to carry out long-term retention studies utilizing carbon 14-labeled sodium formate.

Importance of Studies with Labeled Formate

Buchanan, et al (J.B.C. 173, 69, 1948 and J.B.C. 173, 81, 1948) using C^{13} showed that formate is a rather specific precursor for the 2- and 6- carbon atoms of the purine skeleton of uric acid in pigeons. On the basis of these reports, preliminary investigations have been carried out in this laboratory using C^{14} -labeled sodium formate.

It was observed that on injection of 1.4 μ c of $HC^{14}OONa$ into mice and isolation of viscera nucleic acid purines six hours later the following data were obtained:

<u>Compound</u>	<u>μc Injected</u>	<u>No. of Mice</u>	<u>Specific Activity (μc/mole carbon)</u>		
			<u>Viscera Homogenate</u>	<u>Combined Nucleic Acid</u>	<u>NA Purines</u>
$HC^{14}OONa$	1.4	4	8.48	57.6	138.4
$NaHC^{14}O_2$	11.8	4	1.0	2.5	3.3

This specificity of a carbon atom from a simple, easily prepared, relatively non-toxic compound for a chromosomal fraction offers a means of studying the "Hazards Involved in Use of C^{14} " under most stringent conditions. Using doses of the order of 140 μ c per mouse, it should be possible to obtain chromosome purine activities of about 14,000 μ c/mole of carbon at six hours.

Proposed Program Outline

- A. Long-term over-all body C^{14} retention studies using $HC^{14}OONa$.
- B. Parallel nucleic acid and NA purine turnover studies.
- C. Investigation of acute toxic effects of high levels of carbon 14 (from formate) on mice. Pathological studies on tissues from these mice.

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- D. Studies on the effects of high levels of formate activity on the pattern of deaths from spontaneous leukemia in Alca mice. In this experiment it seems probable that high chromosome nucleic acid activity would provide a situation most conducive to carcinogenic or anti-leukemic action.
- E. Blood smear and marrow autoradiographs ^{will} ~~would~~ be prepared from mice injected with $HC^{14}OONa$ with the objective of obtaining fundamental information on hematopoiesis by following a nucleic acid precursor into nucleated marrow and peripheral blood cells.

Estimated Cost of Program (1 Year)

It is believed that the above program could be carried out for a sum of \$18,000.

The proposed contract amount anticipates direct costs including salaries of technical personnel, expendable supplies, special apparatus, travel expense, and indirect costs (overhead).

Southern Research Institute has conducted research projects for the Office of Naval Research, Navy Bureau of Ordnance, Army Chemical Corps, and the Atomic Energy Commission. Various allowances on indirect costs have applied on these contracts, depending to some extent upon accounting practices of the agency involved. It is therefore assumed that the allowance for indirect costs (as a percentage of direct salary charges) could be negotiated with Atomic Energy Commission representatives at a later date.

Birmingham, Alabama
March 29, 1950
Proposal No. 184
mfm (6) (5)

1641111

SUPPLEMENT TO PROPOSAL NO. 184

SUBMITTED MARCH 29, 1950

1. Title: A study of the hazards involved in use of carbon 14.
2. Institution: Southern Research Institute
3. Leaders: Dr. Howard E. Skipper
Dr. Locke White, Jr.
Dr. L. L. Bennett, Jr.
Dr. Jack H. Mitchell, Jr.
4. Scope and Present Status: Covered in recent proposal (dated March 29, 1950).
5. Outline of Work to be Undertaken: Covered in recent proposal.
6. Materials, Equipment, and Facilities:

Metabolism chambers, special strains of leukemia susceptible mice, oxidation equipment and gas phase counting equipment, and laboratory space are readily available. Radioactive sodium formate and other radioactive compounds to be used in this program will be available from Oak Ridge or as a result of other programs under way at Southern Research Institute.

7. Scientific Personnel: Dr. Howard E. Skipper
Dr. Locke White, Jr.
Dr. L. L. Bennett, Jr.
Dr. Jack H. Mitchell, Jr.
Mrs. Juanita Chapman
Miss Linda Simpson

8. Proposed budget (1 year):

Salaries (including sick leave, vacation
and holiday pay):

Biochemist.....	# 6,380.87
Organic chemist.....	1,063.48
Physical chemist.....	1,063.48
Physicist.....	1,118.70
Biologist.....	<u>2,977.74</u>

12,604.27

Contribution to employees' regular
retirement fund

435.00

Carried forward # 13,039.27

Brought forward \$ 13,039.27

Apparatus and Equipment:

1 - Autoscaler ¹	\$ 650.00	
1 - Windowless flow counter ¹ ...	350.00	
2 - Sample storage cabinet ¹	50.00	
1 - C ¹⁴ reference source ¹	<u>8.00</u>	
		1,058.00

Expendable Supplies:

Radioactive sodium formate ² ...	\$1,500.00	
Other radioactive compounds...	500.00	
Glassware and miscellaneous...	<u>300.00</u>	
		2,300.00

Travel 250.00

Sub-total..... \$ 16,647.27

Overhead at 8% of above costs 1,331.78

Total..... \$ 17,979.05

¹ Necessary to speed up our now rather slow and overtaxed gas phase counting equipment.

² To be purchased from Isotopes Division.

9. Other Responsibilities of Investigators:

Dr. Howard E. Skipper - About 40% of time applied to other technical and administrative tasks.

Dr. Locke White, Jr. (Head of Physics and Physical Chemistry Division) A small amount of Dr. White's time will be applied to this program as required for special calculations and counting techniques.

Dr. L. L. Bennett, Jr. - Organic chemist assigned to synthesis of carbon 14-labeled compounds and preparation of highly active materials for injection. A relatively small amount of Dr. Bennett's time will be applied to this program.

Dr. Jack H. Mitchell, Jr. - Part time efforts on the effects of anti-leukemic agents on nucleoprotein metabolism for Committee on Growth.

Mrs. Janita Chapman, A. B., will be full time on this program.

1111493

Miss Linda Simpson, B.S., will spend about half-time measuring activities on this program and about half-time carrying out similar measurements on an existing cancer program.

Note: It should be pointed out that only technical salaries based on actual time spent on a given project are charged to that project at this institution. Accurate daily time distribution sheets (copy attached) are kept by all technical personnel and charges made accordingly on a pro-rata basis.

Birmingham, Alabama
Supplement to Proposal No. 184
April 27, 1950
mfm (8) (5)

BMB:FBP

23 May 1950

**Dr. Howard E. Skipper
Assistant Director
Southern Research Institute
Birmingham 5, Alabama**

Dear Dr. Skipper:

Your proposal entitled, "Body Retention of Carbon 14," has been reviewed by our staff and I am glad to advise you that this work comes within the interest of the Division of Biology and Medicine.

Since we have previously supported this work through the Office of Naval Research, we feel justified in continuing this project for an additional year. There is no assurance that funds will be available for support at the same level after June 30, 1951.

The technical aspects of this proposal have been approved by the Division of Biology and Medicine, and funds at a level of support as outlined in the proposal have been reserved for it. The negotiation of an appropriate contract is the responsibility of the Commission's Oak Ridge Operations Office. We have forwarded your proposal to them and you should hear from them within the near future. While the contract will be administered by the Oak Ridge Operations Office, the responsibility for the technical aspects of the work rests with the Washington Office.

Sincerely yours,

**Paul B. Pearson
Chief, Biology Branch
Division of Biology and Medicine**

1111496

PROPOSAL FOR A
CONTINUATION OF
THE RESEARCH PROGRAM
ON
BODY RETENTION OF CARBON 14
TO
ATOMIC ENERGY COMMISSION
DIVISION OF BIOLOGY AND MEDICINE

SOUTHERN RESEARCH INSTITUTE

BIRMINGHAM, ALABAMA

MARCH 29, 1950

PROPOSAL NO. 184

11111

BODY RETENTION OF CARBON 14

BACKGROUND

The program underway at Southern Research Institute on the hazard involved in use of carbon 14 has been concerned with:

(1) Quantitative data on the retention of this isotope in various organs and tissues (and the whole body) at extended periods following injection of $\text{NaHC}^{14}\text{O}_3$.

(2) Rate of turnover data on carbon 14 (from $\text{NaHC}^{14}\text{O}_3$) in the whole long bone versus the diaphyseal bone (shaft). These studies involve both dissection and quantitative activity assays and autoradiography.

(3) Investigation of the effect of a given dose (50 mg non-equivalent) of $\text{NaHC}^{14}\text{O}_3$ on the pattern of deaths from leukemia in Aka mice. Similar studies are under way with a C^{14} -labeled compound known to enter into chromosome metabolism, 2,6-diaminopurine.

(4) Study of the over-all distribution and rate of turnover of carbon 14 in certain labeled organic compounds of biological interest (carbonyl and ethoxy-labeled urethan, methylene-labeled ethyl alcohol, urea, 2-labeled 2,6-diaminopurine, and 2-labeled 8-azaguanine).

(5) Experiments having to do with the retention and turnover of $\text{BaC}^{14}\text{O}_3$ when breathed in as an aerosol of the particle size known to be retained in the human lung.

(6) Rate-constant studies on C^{14}O_2 fixation in the carcass and carcass fractions (fat, protein, and glycogen) of mice.

8641111

(7) Fixation of carbon 14 from $\text{NaHC}^{14}\text{O}_3$ and HC^{14}OOH in chromosomal fractions.

Extensive experimental and calculated data have been reported previously on the retention of carbon from labeled sodium bicarbonate. These data which included results up to three months following injection of $\text{NaHC}^{14}\text{O}_3$ were the subject of two recent publications: Studies on the Hazard Involved in Use of C^{14} , I. Retention of Carbon from Labeled Sodium Bicarbonate by Howard E. Skipper, Locke White, Jr., and Carl E. Bryan, J. Biol. Chem. 180, 1187, 1949, and Body Retention of Carbon 14 from Labeled Sodium Bicarbonate, Science 110, 306, 1949, by the same authors. Our further studies on this subject will soon be the subject of another paper to include retention data up to one year following injection of $\text{NaHC}^{14}\text{O}_3$. This publication will emphasize diaphyseal bone carbon 14 turnover versus whole bone carbon turnover.

Another phase of the program has now reached a stage where a formal report in the literature seems indicated. In accordance with the policies of the sponsors encouraging publication, we have prepared and submitted a manuscript entitled "Studies on the Hazard Involved in Use of Carbon 14. II. The Effect of a Single Dose of C^{14} -Labeled Sodium Bicarbonate on the Pattern of Deaths from Spontaneous Leukemia in Akn Mice" by Howard E. Skipper, Martelia J. Bell, and Janita B. Chapman to Cancer Research. This paper will appear in an early issue.

An almost identical experiment is now under way in which Akn mice have been injected with a 5 mc man-equivalent of labeled 2,6-diaminopurine (known to be a precursor of nucleic acid guanine) and set aside to await death from spontaneous leukemia.

111119

Our studies on the over-all distribution and turnover of carbon 14 from labeled organic compounds has progressed to the point where publication of certain of the results seems indicated. A manuscript entitled "Carbonates in the Chemotherapy of Leukemia. VIII. Over-all Tracer Studies on Carbonyl-Labeled Urethan, Ethoxy-Labeled Urethan, and Methylene-Labeled Ethyl Alcohol" by Howard E. Skipper, Leonard L. Bennett, Jr., Carl E. Bryan, Margaret Ann Newton, and Linda Simpson has been prepared.

Experiments having to do with the over-all distribution and turnover of carbon 14 from labeled 2,6-diaminopurine and 8-azaguanine (guanazole) have been carried out.

PROPOSED PROGRAM

In our studies to date we have attempted to emphasize investigation of the most immediate hazards to workers using carbon 14 (tissue and bone radiation following exposure to $C^{14}O_2$ or $BaC^{14}O_3$ aerosol) and for purposes of safety have used relatively low levels of activity and small animals (18 μ c/mouse or a man-equivalent of about 50 mc/man). With the generally encouraging data now at hand regarding safety of experimentation with carbon 14, it is suggested that much more stringent experiments should be carried out to determine what dosages of a seemingly more specific carbon 14-containing compound are required to produce radiation effects.

It is now planned to carry out long-term retention studies utilizing carbon 14-labeled sodium formate.

1111500

Importance of Studies with Labeled Formate

Bushanan, et al (J.B.C. 173, 69, 1948 and J.B.C. 173, 81, 1948) using C¹³ showed that formate is a rather specific precursor for the 2- and 6- carbon atoms of the purine skeleton of uric acid in pigeons. On the basis of these reports, preliminary investigations have been carried out in this laboratory using C¹⁴-labeled sodium formate.

It was observed that on injection of 1.4 µc of HC¹⁴COO_{Na} into mice and isolation of viscera nucleic acid purines six hours later the following data were obtained:

<u>Compound</u>	<u>µc Injected</u>	<u>No. of Mice</u>	<u>Specific Activity (µc/mole carbon)</u>		
			<u>Viscera Homogenate</u>	<u>Combined Nucleic Acid</u>	<u>NA Purines</u>
HC ¹⁴ COO _{Na}	1.4	4	8.48	57.6	138.4
NaHC ¹⁴ O ₂	11.8	4	1.0	2.5	3.3

This specificity of a carbon atom from a simple, easily prepared, relatively non-toxic compound for a chromosomal fraction offers a means of studying the "Hazards Involved in Use of C¹⁴" under most stringent conditions. Using doses of the order of 140 µc per mouse, it should be possible to obtain chromosome purine activities of about 14,000 µc/mole of carbon at six hours.

Proposed Program Outline

- A. Long-term over-all body C¹⁴ retention studies using HC¹⁴COO_{Na}.
- B. Parallel nucleic acid and NA purine turnover studies.
- C. Investigation of acute toxic effects of high levels of carbon 14 (from formate) on mice. Pathological studies on tissues from these mice.

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- D. Studies on the effects of high levels of formate activity on the pattern of deaths from spontaneous leukemia in Akm mice. In this experiment it seems probable that high chromosome nucleic acid activity would provide a situation most conducive to carcinogenic or anti-leukemic action.
- E. Blood smear and marrow autoradiographs would be prepared from mice injected with $HC^{14}OONa$ with the objective of obtaining fundamental information on hematopoiesis by following a nucleic acid precursor into nucleated marrow and peripheral blood cells.

Estimated Cost of Program (1 Year)

It is believed that the above program could be carried out for a sum of \$18,000.

The proposed contract amount anticipates direct costs including salaries of technical personnel, expendable supplies, special apparatus, travel expense, and indirect costs (overhead).

Southern Research Institute has conducted research projects for the Office of Naval Research, Navy Bureau of Ordnance, Army Chemical Corps, and the Atomic Energy Commission. Various allowances on indirect costs have applied on these contracts, depending to some extent upon accounting practices of the agency involved. It is therefore assumed that the allowance for indirect costs (as a percentage of direct salary charges) could be negotiated with Atomic Energy Commission representatives at a later date.

Birmingham, Alabama
March 29, 1950
Proposal No. 184
mfu (6) (5)

SUPPLEMENT TO PROPOSAL NO. 181

SUBMITTED MARCH 29, 1950

1. Title: A study of the hazards involved in use of carbon 14.
2. Institution: Southern Research Institute
3. Leaders: Dr. Howard E. Skipper
Dr. Locke White, Jr.
Dr. L. L. Bennett, Jr.
Dr. Jack H. Mitchell, Jr.
4. Scope and Present Status: Covered in recent proposal (dated March 29, 1950).
5. Outline of Work to be Undertaken: Covered in recent proposal.
6. Materials, Equipment, and Facilities:

Metabolism chambers, special strains of leukemia susceptible mice, oxidation equipment and gas phase counting equipment, and laboratory space are readily available. Radioactive sodium formate and other radioactive compounds to be used in this program will be available from Oak Ridge or as a result of other programs under way at Southern Research Institute.

7. Scientific Personnel: Dr. Howard E. Skipper
Dr. Locke White, Jr.
Dr. L. L. Bennett, Jr.
Dr. Jack H. Mitchell, Jr.
Mrs. Juanita Chapman
Miss Linda Simpson

8. Proposed budget (1 year):

Salaries (including sick leave, vacation
and holiday pay):

Biochemist.....	\$ 6,380.87
Organic chemist.....	1,063.48
Physical chemist.....	1,063.48
Physicist.....	1,118.70
Biologist.....	<u>2,977.74</u>

\$ 12,604.27

Contribution to employees' regular
retirement fund

435.00

Carried forward \$ 13,039.27

SOUTHERN RESEARCH INSTITUTE

1111503

Brought forward \$ 13,039.27

Apparatus and Equipment:

1 - Autosealer ¹	\$ 650.00	
1 - Windowless flow counter ¹ ...	350.00	
2 - Sample storage cabinet ¹	50.00	
1 - C ¹⁴ reference source ¹	<u>8.00</u>	
		1,058.00

Expendable Supplies:

Radioactive sodium formate ² ...	\$1,500.00	
Other radioactive compounds...	500.00	
Glassware and miscellaneous...	<u>300.00</u>	
		2,300.00

Travel 250.00

Sub-total..... \$ 16,647.27

Overhead at 8% of above costs 1,331.78

Total..... \$ 17,979.05

- ¹ Necessary to speed up our now rather slow and overtaxed gas phase counting equipment.
- ² To be purchased from Isotopes Division.

9. Other Responsibilities of Investigators:

Dr. Howard E. Skipper - About 40% of time applied to other technical and administrative tasks.

Dr. Locke White, Jr. (Head of Physics and Physical Chemistry Division) A small amount of Dr. White's time will be applied to this program as required for special calculations and counting techniques.

Dr. L. L. Bennett, Jr. - Organic chemist assigned to synthesis of carbon 14-labeled compounds and preparation of highly active materials for injection. A relatively small amount of Dr. Bennett's time will be applied to this program.

Dr. Jack H. Mitchell, Jr. - Part time efforts on the effects of anti-leukemic agents on nucleoprotein metabolism for Committee on Growth.

Mrs. Juanita Chapman, A. B., will be full time on this program.

SOUTHERN RESEARCH INSTITUTE

1111504

Miss Linda Simpson, B.S., will spend about half-time measuring activities on this program and about half-time carrying out similar measurements on an existing cancer program.

Note: It should be pointed out that only technical salaries based on actual time spent on a given project are charged to that project at this institution. Accurate daily time distribution sheets (copy attached) are kept by all technical personnel and charges made accordingly on a pro-rata basis.

1111505

Birmingham, Alabama
Supplement to Proposal No. 184
April 27, 1950
mfm (8) (5)

BMB:FBP

23 May 1950

**Dr. Edward E. Skipper
Assistant Director
Southern Research Institute
Birmingham 5, Alabama**

Dear Dr. Skipper:

Your proposal entitled, "Body Retention of Carbon 14," has been reviewed by our staff and I am glad to advise you that this work comes within the interest of the Division of Biology and Medicine.

Since we have previously supported this work through the Office of Naval Research, we feel justified in continuing this project for an additional year. There is no assurance that funds will be available for support at the same level after June 30, 1951.

The technical aspects of this proposal have been approved by the Division of Biology and Medicine, and funds at a level of support as outlined in the proposal have been reserved for it. The negotiation of an appropriate contract is the responsibility of the Commission's Oak Ridge Operations Office. We have forwarded your proposal to them and you should hear from them within the near future. While the contract will be administered by the Oak Ridge Operations Office, the responsibility for the technical aspects of the work rests with the Washington Office.

Sincerely yours,

**Paul E. Pearson
Chief, Biology Branch
Division of Biology and Medicine**

1111507

UNITED STATES ATOMIC ENERGY COMMISSION
DIVISION OF BIOLOGY AND MEDICINE
WASHINGTON, D. C.

TO : A. E. Holland, Jr. Chief, DATE: 23 May 1950
Medicine and Research Division, Oak Ridge
FROM : Paul B. Pearson, Chief, Biology Branch
Division of Biology and Medicine
SUBJECT: TRANSMITTAL OF RESEARCH PROPOSAL FOR CONTRACT NEGOTIATION

REFER TO

SYMBOL: BEB:PBP

This letter with enclosures, in triplicate, is sent in accordance with the procedure described in a letter from the General Manager to all Managers of Operations dated January 27, 1949.

1. Institution: Southern Research Institute
Birmingham, Alabama
2. Investigator(s): Howard E. Skipper
3. Title: "Body Retention of Carbon 14"
4. Duration - From: July 1, 1950 To: 1 year from date of contract
5. AEC Technical Supervision: Division of Biology and Medicine
Biology Activity #6400
6. Recommended Support: \$17,979 (including 8% overhead)
7. Other Comments

This is a continuation of a project that the AEC has supported through the Office of Naval Research.

PROPOSAL FOR A
CONTINUATION OF
THE RESEARCH PROGRAM
ON
BODY RETENTION OF CARBON 14
TO
ATOMIC ENERGY COMMISSION
DIVISION OF BIOLOGY AND MEDICINE

SOUTHERN RESEARCH INSTITUTE

BIRMINGHAM, ALABAMA

MARCH 29, 1950

PROPOSAL NO. 184

1111510

BODY RETENTION OF CARBON 14

BACKGROUND

The program underway at Southern Research Institute on the hazard involved in use of carbon 14 has been concerned with:

(1) Quantitative data on the retention of this isotope in various organs and tissues (and the whole body) at extended periods following injection of $\text{NaHC}^{14}\text{O}_3$.

(2) Rate of turnover data on carbon 14 (from $\text{NaHC}^{14}\text{O}_3$) in the whole long bone versus the diaphyseal bone (shaft). These studies involve both dissection and quantitative activity assays and autoradiography.

(3) Investigation of the effect of a given dose (50 mc man-equivalent) of $\text{NaHC}^{14}\text{O}_3$ on the pattern of deaths from leukemia in Akm mice. Similar studies are under way with a C^{14} -labeled compound known to enter into chromosome metabolism, 2,6-diaminopurine.

(4) Study of the over-all distribution and rate of turnover of carbon 14 in certain labeled organic compounds of biological interest (carbonyl and ethoxy-labeled urethan, methylene-labeled ethyl alcohol, urea, 2-labeled 2,6-diaminopurine, and 2-labeled 8-azaguanine).

(5) Experiments having to do with the retention and turnover of $\text{BaC}^{14}\text{O}_3$ when breathed in as an aerosol of the particle size known to be retained in the human lung.

(6) Rate-constant studies on C^{14}O_2 fixation in the carcass and carcass fractions (fat, protein, and glycogen) of mice.

(7) Fixation of carbon 14 from $\text{NaHC}^{14}\text{O}_3$ and HC^{14}OOH in chromosomal fractions.

Extensive experimental and calculated data have been reported previously on the retention of carbon from labeled sodium bicarbonate. These data which included results up to three months following injection of $\text{NaHC}^{14}\text{O}_3$, were the subject of two recent publications: Studies on the Hazard Involved in Use of C^{14} . I. Retention of Carbon from Labeled Sodium Bicarbonate by Howard E. Skipper, Locke White, Jr., and Carl E. Bryan, J. Biol. Chem. 180, 1187, 1949, and Body Retention of Carbon 14 from Labeled Sodium Bicarbonate, Science 110, 306, 1949, by the same authors. Our further studies on this subject will soon be the subject of another paper to include retention data up to one year following injection of $\text{NaHC}^{14}\text{O}_3$. This publication will emphasize diaphyseal bone carbon 14 turnover versus whole bone carbon turnover.

Another phase of the program has now reached a stage where a formal report in the literature seems indicated. In accordance with the policies of the sponsors encouraging publication, we have prepared and submitted a manuscript entitled "Studies on the Hazard Involved in Use of Carbon 14. II. The Effect of a Single Dose of C^{14} -Labeled Sodium Bicarbonate on the Pattern of Leukemia from Spontaneous Leukemia in Akra Mice" by Howard E. Skipper, Martelia J. Bell, and Juanita B. Chapman to Cancer Research. This paper will appear in an early issue.

An almost identical experiment is now under way in which Akra mice have been injected with a 5 mc man-equivalent of labeled 2,6-diaminopurine (known to be a precursor of nucleic acid guanine) and set aside to await death from spontaneous leukemia.

1111512

Our studies on the over-all distribution and turnover of carbon 14 from labeled organic compounds has progressed to the point where publication of certain of the results seems indicated. A manuscript entitled "Carbamates in the Chemotherapy of Leukemia. VIII. Over-all Tracer Studies on Carbonyl-Labeled Urethan, Ethoxy-Labeled Urethan, and Methylene-Labeled Ethyl Alcohol" by Howard E. Skipper, Leonard L. Bennett, Jr., Carl E. Bryan, Margaret Ann Newton, and Linda Simpson has been prepared.

Experiments having to do with the over-all distribution and turnover of carbon 14 from labeled 2,6-diaminopurine and 8-azaguanine (guanazole) have been carried out.

PROPOSED PROGRAM.

In our studies to date we have attempted to emphasize investigation of the most immediate hazards to workers using carbon 14 (tissue and bone radiation following exposure to $C^{14}O_2$ or $BaC^{14}O_3$ aerosol) and for purposes of safety have used relatively low levels of activity and small animals (18 μ c/mouse or a man-equivalent of about 50 μ c/man). With the generally encouraging data now at hand regarding safety of experimentation with carbon 14, it is suggested that much more stringent experiments should be carried out to determine what dosages of a seemingly more specific carbon 14-containing compound are required to produce radiation effects.

It is now planned to carry out long-term retention studies utilizing carbon 14-labeled sodium formate.

Importance of Studies with Labeled Formate

Buchanan, et al (J.B.C. 173, 69, 1948 and J.B.C. 173, 81, 1948) using C¹³ showed that formate is a rather specific precursor for the 2- and 6- carbon atoms of the purine skeleton of uric acid in pigeons. On the basis of these reports, preliminary investigations have been carried out in this laboratory using C¹⁴-labeled sodium formate.

It was observed that on injection of 1.4 µc of HC¹⁴COONa into mice and isolation of viscera nucleic acid purines six hours later the following data were obtained:

<u>Compound</u>	<u>µc Injected</u>	<u>No. of Mice</u>	<u>Specific Activity (µc/mole carbon)</u>		
			<u>Viscera Homogenate</u>	<u>Combined Nucleic Acid</u>	<u>NA Purines</u>
HC ¹⁴ COONa	1.4	4	8.48	57.6	138.4
NaHC ¹⁴ O ₂	11.8	4	1.0	2.5	3.3

This specificity of a carbon atom from a simple, easily prepared, relatively non-toxic compound for a chromosomal fraction offers a means of studying the "Hazards Involved in Use of C¹⁴" under most stringent conditions. Using doses of the order of 140 µc per mouse, it should be possible to obtain chromosome purine activities of about 14,000 µc/mole of carbon at six hours.

Proposed Program Outline

- A. Long-term over-all body C¹⁴ retention studies using HC¹⁴COONa.
- B. Parallel nucleic acid and NA purine turnover studies.
- C. Investigation of acute toxic effects of high levels of carbon 14 (from formate) on mice. Pathological studies on tissues from these mice.

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- D. Studies on the effects of high levels of formate activity on the pattern of deaths from spontaneous leukemia in Alka mice. In this experiment it seems probable that high chromosome nucleic acid activity would provide a situation most conducive to carcinogenic or anti-leukemic action.
- E. Blood smear and marrow autoradiographs would be prepared from mice injected with $HC^{14}OONa$ with the objective of obtaining fundamental information on hematopoiesis by following a nucleic acid precursor into nucleated marrow and peripheral blood cells.

Estimated Cost of Program (1 Year)

It is believed that the above program could be carried out for a sum of \$18,000.

The proposed contract amount anticipates direct costs including salaries of technical personnel, expendable supplies, special apparatus, travel expense, and indirect costs (overhead).

Southern Research Institute has conducted research projects for the Office of Naval Research, Navy Bureau of Ordnance, Army Chemical Corps, and the Atomic Energy Commission. Various allowances on indirect costs have applied on these contracts, depending to some extent upon accounting practices of the agency involved. It is therefore assumed that the allowance for indirect costs (as a percentage of direct salary charges) could be negotiated with Atomic Energy Commission representatives at a later date.

Birmingham, Alabama
March 29, 1950
Proposal No. 184
sm (6) (5)

1111515

SUPPLEMENT TO PROPOSAL NO. 184

SUBMITTED MARCH 29, 1950

1. Title: A study of the hazards involved in use of carbon 14.
2. Institution: Southern Research Institute
3. Leaders: Dr. Howard E. Skipper
Dr. Locke White, Jr.
Dr. L. L. Bennett, Jr.
Dr. Jack H. Mitchell, Jr.
4. Scope and Present Status: Covered in recent proposal (dated March 29, 1950).
5. Outline of Work to be Undertaken: Covered in recent proposal.
6. Materials, Equipment, and Facilities:

Metabolism chambers, special strains of leukemia susceptible mice, oxidation equipment and gas phase counting equipment, and laboratory space are readily available. Radioactive sodium formate and other radioactive compounds to be used in this program will be available from Oak Ridge or as a result of other programs under way at Southern Research Institute.

7. Scientific Personnel: Dr. Howard E. Skipper
Dr. Locke White, Jr.
Dr. L. L. Bennett, Jr.
Dr. Jack H. Mitchell, Jr.
Mrs. Juanita Chapman
Miss Linda Simpson

8. Proposed budget (1 year):

Salaries (including sick leave, vacation
and holiday pay):

Biochemist.....	\$ 6,380.87
Organic chemist.....	1,063.48
Physical chemist.....	1,063.48
Physicist.....	1,118.70
Biologist.....	<u>2,977.74</u>

\$ 12,604.27

Contribution to employees' regular
retirement fund

135.00

Carried forward \$ 13,039.27

Brought forward \$ 13,039.27

Apparatus and Equipment:

1 - Autoscaler ¹	\$ 650.00	
1 - Windowless flow counter ¹ ...	350.00	
2 - Sample storage cabinet ¹	50.00	
1 - C ¹⁴ reference source ¹	<u>8.00</u>	
		1,058.00

Expendable Supplies:

Radioactive sodium formate ²	\$1,500.00	
Other radioactive compounds...	500.00	
Glassware and miscellaneous...	<u>300.00</u>	
		2,300.00

Travel 250.00

Sub-total..... \$ 16,647.27

Overhead at 8% of above costs 1,331.78

Total..... \$ 17,979.05

¹ Necessary to speed up our now rather slow and overtaxed gas phase counting equipment.

² To be purchased from Isotopes Division.

9. Other responsibilities of Investigators:

Dr. Howard B. Skipper - About 40% of time applied to other technical and administrative tasks.

Dr. Locke White, Jr. (head of Physics and Physical Chemistry Division) A small amount of Dr. White's time will be applied to this program as required for special calculations and counting techniques.

Dr. L. L. Bennett, Jr. - Organic chemist assigned to synthesis of carbon 14-labeled compounds and preparation of highly active materials for injection. A relatively small amount of Dr. Bennett's time will be applied to this program.

Dr. Jack H. Mitchell, Jr. - Part time efforts on the effects of anti-leukemic agents on nucleoprotein metabolism for committee on Growth.

Mrs. Juanita Chapman, A. B., will be full time on this program.

111517

Miss Linda Simpson, B.S., will spend about half-time measuring activities on this program and about half-time carrying out similar measurements on an existing cancer program.

Note: It should be pointed out that only technical salaries based on actual time spent on a given project are charged to that project at this institution. Accurate daily time distribution sheets (copy attached) are kept by all technical personnel and charges made accordingly on a pro-rata basis.

1111518

Birmingham, Alabama
Supplement to proposal No. 124
April 27, 1950
afa (8) (5)

E R:RFP

23 May 1950

Dr. Edward E. Skipper
Assistant Director
Southern Research Institute
Birmingham 5, Alabama

Dear Dr. Skipper:

Your proposal entitled, "Body Retention of Carbon 14," has been reviewed by our staff and I am glad to advise you that this work comes within the interest of the Division of Biology and Medicine.

Since we have previously supported this work through the Office of Naval Research, we feel justified in continuing this project on a similar basis. There is a possibility that funds will be available for support at the same level after June 30, 1951.

The technical aspects of this proposal have been approved by the Division of Biology and Medicine, and funds at a level of support as outlined in the proposal have been reserved for it. The negotiation of an appropriate contract is the responsibility of the Commission's Oak Ridge Operations Office. We have forwarded an invitation to the Oak Ridge Office and you should hear from them within the near future. While the contract will be administered by the Oak Ridge Operations Office, the responsibility for the technical aspects of the work rests with the Washington Office.

Sincerely yours,

Paul E. Pearson
Chief, Biology Branch
Division of Biology and Medicine

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