

Modification 1, dated June 6, 1958 and effective
as of June 1, 1958, to Contract AT-(40-1)-1829

The University of Tennessee

Distributed: June 6, 1958

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AT-(40-1)-1829- Univ. of Tenn.

ACC:A:3

Cak Ridge, Tennessee
June 6, 1958

The University of Tennessee
Office of the Secretary
Knoxville, Tennessee

Attention: Mr. J. P. Hess

Subject: MODIFICATION NO. 1 TO CONTRACT NO. AT-(40-1)-1329

Gentlemen:

Enclosed, for your retention, are one duly signed copy and one
conformed copy of Modification No. 1 to Contract No. AT-(40-1)-1329.

Very truly yours,

John R. Moore
Director, Contract Division
Cak Ridge Operations

✓ NHT
Enclosure:
Mod. 1 (2 copies)

cc: Fr. Frederick W. Lengenann, w/cy encl
The University of Tennessee
School of Biological Sciences
Division of Chemistry
Memphis 3, Tennessee

OFFICE ▶	Contract Div.	Director Contract Div.				
SURNAME ▶	<i>Brown</i> Brown:arb	<i>JRM</i> JRMoore				
DATE ▶	6-6-58	6-6-58				

1108684

THE UNIVERSITY OF TENNESSEE
KNOXVILLE
OFFICE OF THE SECRETARY

June 4, 1958

Mr. John R. Moore
Director, Contract Division
Oak Ridge Operations
U. S. Atomic Energy Commission
Oak Ridge, Tennessee

Dear Mr. Moore:

Enclosed herewith are two copies of Modification No. 1 to Contract No. AT-(40-1)-1829 between the Atomic Energy Commission and The University of Tennessee Medical Units at Memphis, Tennessee. This modification has been signed for the University by President C. E. Brehm.

When signed for the Atomic Energy Commission, kindly see that one fully executed and one conformed copy of this modification is returned to this office for the document files of the University.

Very truly yours,


J. P. Hess

es

Enclosures - 2

1108685

9
6-3

L. P. Mackay, Director
Finance Division

June 3, 1959

Norman W. Beth, Director
Research and Development Division

CONTRACT NO. AT-(20-1)-1929 - UNIVERSITY OF TENNESSEE

SYMBOL: OFC:JTB

This is to advise you that work under Contract No. AT-(20-1)-1929 with the University of Tennessee has been completed and that an acceptable final report has been submitted. Therefore, the obligations of the University have been concluded and the contract should be closed out.

Norman W. Beth
Director
Research and Development Division

W. J. Burlison
Director
Finance Division

Res. Ser. Br. Bio. Br. Res. & Dev.

Burlison:ja

1108686

THE UNIVERSITY OF TENNESSEE

SCHOOL OF BIOLOGICAL SCIENCES

MEMPHIS 3, TENNESSEE

DIVISION OF CHEMISTRY

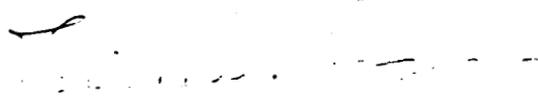
May 11, 1958

Mr. John R. Moore, Director
Contract Division
Oak Ridge Operations
Atomic Energy Commission
Oak Ridge, Tennessee

Dear Mr. Moore,

Enclosed you will find the completed summary form
for my project Contract No. AT-(18-1)-1126. The signed copies of
the contract modification will be sent to you under separate cover
from Knoxville.

Sincerely yours,


Frederick W. Lenzemann

1108687

AC:ARB

Oak Ridge, Tennessee
May 12, 1958

The University of Tennessee
College of Medicine
Division of Chemistry
Memphis, Tennessee

Attention: Dr. Frederick W. Langmann

Subject: MODIFICATION NO. 1 TO CONTRACT NO. AT-(40-1)-1829

Gentlemen:

Enclosed, in triplicate, is proposed Modification No. 1 to the subject contract which provides for additional research and extension of the period, to May 31, 1959, during which the work under the contract may be conducted. Also enclosed is a Notice of Research Project form which is to be used in submitting your summary statement.

If the modification, as submitted, is satisfactory, will you kindly sign two of the enclosed copies in the space provided for the Senior Investigator, have such two copies signed by the proper official of the University and return them to this office. The remaining copy is for your retention. After signature on behalf of the Commission, one duly signed copy of the modification will be returned for your files.

The completed summary statement, with the date of its submittal shown thereon, should be returned with the signed copies of the modification.

Very truly yours,

John R. Moore
Director, Contract Division
Oak Ridge Operations

- ARB*
- Enclosures:
 1. Proposed Mod. 1 (in trip.)
 2. Notice of Research Project Form

BCC: Div. of Biology & Medicine, AEC Headquarters

OFFICE ▶	C. S. Shoup	Contract Div.	Director	Contract Div.	
SURNAME ▶		<i>Brown</i> Brown:arb	<i>JR Moore</i> JRM:moore		
DATE ▶		5-13-58	5-13-58		

1108688

J. W. Ould, Jr., Assistant General Counsel

April 29, 1958

John R. Moore, Director, Contract Division

PREPARATION OF MODIFICATION TO CONTRACT AT-(40-1)-1829 WITH UNIVERSITY OF TENNESSEE, MEMPHIS, TENNESSEE

SYMBOL: ACD:EM

Mod. 1

Please prepare an appropriate modification to the subject contract to provide for additional research to be completed not later than May 31, 1959, with new funds in the amount of \$8,580. This action is covered by Contract Authorization No. W-58-383, dated April 21, 1958, in the amount of \$8,580.

A Request for Contract Action dated April 25, 1958, from the Research and Development Division, is enclosed for your use.

John R. Moore

- Enclosures, *RF*
1. Request for Cont. Action
 2. Budget
 3. Res'nc'
 4. Cont. Auth.
 5. Renewal Proposal

cc/ L. D. McKay
 C. S. Sheup
 A. Brown, w/encls. 1, 2 & 4

Dep. Director, Director,

OFFICE	Contract Div.	Contract Div.			
PERSON	<i>[Signature]</i>	John R. Moore			
DATE	4-29-58	4-29-58			

1108689

1/28/58

Memphis, Tennessee
January 28, 1958

*H. H. H. H.
Alice*

Dr. F. W. Lengemann
Division of Chemistry
University of Tennessee
Memphis 3, Tennessee

Subject: CONTRACT NO. AT-(40-1)-1829

Dear Dr. Lengemann:

Reference is made to your letter of January 21, 1958, requesting permission to transfer approximately 10% of the funds provided in the budget of contract no. AT-(40-1)-1829 under the category "supplies" to the "Travel" category in order for you to attend the Federation meetings at Philadelphia.

This is to advise you that we offer no objection to the transfer of funds, as requested, provided that no additional funds will be required from the AEC for the current period as a result of this transfer.

Your cooperation is appreciated.

Very truly yours,

C. S. Group
Chief, Biology Branch
Research and Development Division

cc: C. S. Hipton, Memphis
J. R. Moore ✓

Res. Ser. Br. Bio. Br.

Burleson:ja

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ACC:ARB

Oak Ridge, Tennessee
June 7, 1957

The University of Tennessee
Office of the Secretary
Knoxville, Tennessee

Attention: Mr. J. P. Hess

Subject: CONTRACT NO. AT-(40-1)-1829

Gentlemen:

Enclosed, as requested in your letter of June 4, 1957, you will find one duly signed copy of the subject contract and one conformed copy thereof.

Very truly yours,

active

John R. Moore
Director, Contract Division
Oak Ridge Operations

✓
Enclosure: (in dup.)
Cont. 1829

OFFICE ▶	Contract Div.	Actg Director Contract Div.				
SURNAME ▶	<i>Brown</i> Brown:arb	<i>RG</i> RG:humphries				
DATE ▶	6-7-57	6-7-57				

1108691

THE UNIVERSITY OF TENNESSEE
KNOXVILLE
OFFICE OF THE SECRETARY

June 4, 1957

Mr. John R. Moore
Director, Contract Division
Oak Ridge Operations
Atomic Energy Commission
Oak Ridge, Tennessee

Dear Mr. Moore:

Returned herewith are two copies of Contract No. AT-(40-1)-1829 between The University of Tennessee and the Atomic Energy Commission covering research at the Medical Units.

Both copies have been signed by President C. E. Brehm and the Seal has been affixed and attested by the writer. When signed by the Atomic Energy Commission, will you kindly return one fully executed copy and one conformed copy to this office.

Very truly yours,


J. P. Hess

ebs

Enclosures - 2

cc-Mr. Cecil Q. Tipton

1108692

Contract AT-(40-1)-1829

The University of Tennessee

Distributed: June 7, 1957

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Contract Files (2)

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Extra Copies

1108693

In Reply
Refer To: ACC:JEB

Cox Ridge, Tennessee
May 16, 1957

University of Tennessee
College of Medicine
Division of Chemistry
School of Biological Sciences
Memphis, Tennessee

Attention: Dr. Frederick W. Langemann

Subject: CONTRACT NO. AT-(40-1)-1829

Gentlemen:

Your research project, which was submitted to the Commission's Division of Biology and Medicine, Washington, D. C., has been approved by that office in the amount of \$8,640.00 and has been forwarded to this office for preparation of an appropriate contract covering the Commission's support of your project.

Enclosed are three copies of a contract, numbered as shown in the above subject, which incorporates in Appendix "A" a description of your project and the budget for the initial period which you are to follow as a general guide. Also enclosed is a Notice of Research Project form which is to be used in submitting your summary statement.

It is requested that you sign two copies of the contract in the space provided for the Senior Investigator and have such two copies signed by the proper official of the University, returning both copies to this office. The remaining copy is for your retention. The summary statement, with the date of its submittal shown thereon, should be returned along with the signed copies of the contract. After signature on behalf of the Commission, one duly signed copy of the contract will be returned for your retention.

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 45 per cent of 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. Another

1108694

May 16, 1957

45 per cent of the agreed compensation will be paid to you within six months from the date of the first payment. The remaining 10 per cent of the agreed compensation will be paid to you upon receipt and acceptance of a satisfactory progress report, or the final report, as the case may be.

To assist you in preparing an appropriate voucher, there is enclosed an instruction sheet containing numbered instructions corresponding with numbers appearing on a specimen copy of the voucher form. Vouchers should be submitted to the Research and Development Division, Oak Ridge Operations Office, U. S. Atomic Energy Commission, Post Office Box 8, Oak Ridge, Tennessee, in one original (white) and four copies (yellow). 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. 3 which requires the submission of a 200-word summary statement describing the purpose and scope of your project. This 200-word summary statement should show the date of its submittal.

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, I will be glad to furnish you with additional information.

Very truly yours,

Signed by R. G. Humphries *R. G. Humphries*

John R. Moore
Director, Contract Division
Oak Ridge Operations

Enclosures:

- 1. Cont. AT-(40-1)-1829 (3 cys.)
- 2. Notice of Research Project Form
- 3. Vouchers and Instruction Sheets

BCC: Division of Biology & Medicine,
Washington, D. C.
C. S. Shoup

OFFICE ▶	Contract Div.	Actg Director Contract Div.			
SURNAME ▶	<i>Brown:arb</i>	<i>RGHumphries</i>			
DATE ▶	5-16-57	5-16-57			

1108695

TO : Addressees Listed Below

DATE: May 7, 1958

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 NO. 1 - CONTRACT AT-(LC-1)-1929

UNIVERSITY OF TENNESSEE

Addressees:	Division:	Init:	Date:	Remarks:
1. B. G. Huddles	Contracts	BMH	5/7/58	
2. A. B. Miller	Budget	arm	5/7/58	
3. C. S. Shoup	Res. & Dev.	gsl ESS	5/9/58 5/9/58	
4. I. D. Mackay	Finance	shy A		
5. -				
6. -				

RETURN TO: . Proc., Contract Div. - 4719

/o
5/12/58

Office Memorandum • UNITED STATES GOVERNMENT

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

DATE: April 2, 1958

FROM : John L. Moore, Director, Contract Division

SUBJECT: PREPARATION OF MODIFICATION TO CONTRACT AT-(40-1)-1329 WITH
UNIVERSITY OF TENNESSEE, MEMPHIS, TENNESSEE

SYMBOL: ACD:BSH

Please prepare an appropriate modification to the subject contract to provide for additional research to be completed not later than May 31, 1959, with new funds in the amount of \$8,530. This action is covered by Contract Authorization No. RM-58-383, dated April 21, 1958, in the amount of \$8,530.

A Request for Contract Action dated April 25, 1958, from the Research and Development Division, is enclosed for your use.

John L. Moore

Enclosures:

1. Request for Cont. Action
2. Budget
3. Resume
4. Cont. Auth.
5. Renewal Proposal

cc: I. D. McKay
C. S. Shoup
A. Brown, w/encls. 1, 2 & 4

1. TO: 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 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)-1829 Contractor: University of Tennessee Memphis, Tennessee. Review and approval of Contract, Subcontract 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 \$ 5,580.00

Source of Funds

Approved ORO Financial Plan, Quarter, Fiscal Year 19 Project No. or, Activity No. Funds to be Obligated: Allotment No. Procurement Directive No. Issuing Office

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:)

<p>6. <u>Contract Board Docket</u> No. _____ (To be assigned by Board Secretary)</p>	<p>7. <u>Request Submitted By: (signed)</u> Date: _____ 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: "The Metabolism of Alkaline Earth Metals by Bone" (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 additional research to be completed not later than May 31, 1959, with new funds in the amount of \$8,580. (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. Snilling to S. R. Sapirie dated April 21, 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 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
 4-21-59

Budget for Contract No. AP-(40-1)-1-29

17	<u>Salaries and Wages:</u>		87,794.00
	Dr. F. W. Lengemann (30% of time)	\$1,500.00	
	Consultants	1,100.00	
	Research Technicians	4,294.00	
	Administrative and Secretarial Assistance	500.00	
21	<u>Equipment:</u>		1,700.00
22	<u>Supplies:</u>		1,600.00
23	<u>Travel:</u>		100.00
24	<u>Publication and Communication:</u>		100.00
25	<u>Overhead and Indirect Costs:</u>		1,512.00
			<hr/>
		Total	92,896.00*

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

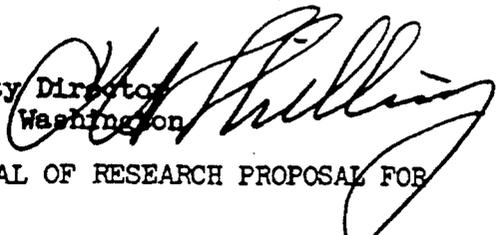
*Recognize that the project may receive additional financial support as described in the proposal.

1108700

UNITED STATES ATOMIC ENERGY COMMISSION
WASHINGTON, D. C.

Contract Authorization No. EM-58-383
APR 21 1958

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

FROM : Charles 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 : **EMM:JFB**

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: The University of Tennessee
- ✓ 2. Investigator (s): Frederick W. Lengemann
- ✓ 3. Title: "The Metabolism of Alkaline Earth Metals by Bone"

4. () New Contract, (x) Renewal of Contract No. AT(40-1)1829
5. Duration: 6/1/58 through 5/31/59
6. AEC Technical Representative: John F. Bonner 
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>
✓ <u>06-81-91 (24)</u>	<u>6220</u>	<u> </u>	<u>\$ 8,580</u>	<u>\$ 8,580</u>
<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>

11 3851

APR 25 1958

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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:
- () 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:

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: () 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 March 1, 1958
 () "B" - Notification letter, dated APR 2 - 1958
 () "C" - Other correspondence, none 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.)	
Program Analysis Branch:	Branch File: White copy (w encl.)
White copy (w/o encl.)	

Renewal Proposal to the A.E.C.

Title: The Metabolism of Alkaline Earth Metals by Bone

Contract No. AT - (40-1) - 1829

For the period June 1, 1958 through May 31, 1959

Scientific Background

A review of some of the aspects of alkaline earth metabolism by bone has been presented previously in the original project proposal submitted to the A.E.C.. Since that time little new evidence has appeared in the literature to elucidate the mechanisms of deposition in and removal of these elements from bone. Therefore, a review of the literature will not be attempted.

However, recently a good deal of work has appeared concerning the metabolism of strontium-90 by humans and laboratory animals. A report of the Sr-90 levels in human bone has been published by Eckelmann, Kulp, and Schulert (1) and a review of the known selective processes has been compiled by Comar, Russell, and Wasserman (2). Comar implicates the intestine and the kidney as being the major sites of discrimination against strontium present in the diet. Bone is not considered to be important. However, he reports that the new born human shows a greater selection against strontium-90 than can be accounted for by the known selective processes of the maternal intestine, kidney, and placenta. It would seem possible that here is the place where the selective processes of bone would play an important role. Also, once strontium-90 is deposited in bone, the discriminatory ability of bone would be important for its removal. To test the ability of human bone to differentiate between strontium and calcium is difficult. However, some evidence has been obtained that selection may occur during the resorption phase of bone metabolism of human bone and is presented in the accompanying progress report. More evidence is needed to confirm this.

1108703

References

1. Eckelmann, W. R., J. L. Kulp, and A. R. Schulert
Sci. 127: 266 - 274 (1958)
2. Comar, C. L., R. S. Russell, and R. H. Wasserman
Sci. 126: 485 - 492 (1957)

Scientific Scope

As stated in the original proposal the major objectives of the research program are:

1. To compare the metabolism by bone of the members of the alkaline earth group of elements.
2. To investigate the phases of mineral metabolism by bone to determine what processes are enzyme influenced.
3. To determine the rates of strontium and calcium removal and to attempt to alter them.
4. To describe the mechanism by which stable strontium inhibits calcification and increases the discrimination against radioactive strontium.

The accompanying progress report details the results obtained to date in fulfilling these objectives. More work remains to be done. The following program is planned for the next fiscal year.

1. The Uptake of Strontium and Calcium by Human Bone

Some data has been obtained but more evidence is needed before conclusions can be made. To this end, an effort will be made to secure more fetuses and to culture the bones obtained. The Sr/Ca ratio at equilibrium and during removal can be found conveniently by the methods now in use. The amount of work done will depend upon the supply and cooperation received. If sufficient numbers are forthcoming, other alkaline earths may be investigated.

Continuing attempts will be made to obtain human periosteum and to grow it in tissue culture to obtain bone.

2. The Effect of Species upon Strontium Uptake

The work to date indicates that the medium does not function in the selection but that the mechanism is contained within the bones. The variation noted between embryonic chick and human bone suggests that species do vary in their selective ability. To investigate this the Sr/Ca ratios at equilibrium

and during removal will be investigated for the bones of as many species as can be obtained. If the results indicate a species difference, other alkaline earths will be investigated.

3. The Effect of the Degree of Calcification upon Discrimination

In the studies made thus far, it has been noted that the ends of the bones selected for calcium and against strontium more than did the shafts. This was observed for both human and chick bone. Also, the human bones were more calcified than the chick bones and showed much different Sr/Ca ratios. To investigate this tibias from 7-9-12- and 14-day old embryos will be cultivated for 7 days with activity in the medium. At this time one-half of the bones will be removed and assayed while the remainder will be shifted to non-labeled medium for four days before being assayed. Thus, the effect of the degree of calcification on the equilibrium ratio and removal ratio should be obtained.

If human embryos can be obtained the wide variation in their ages should make comparisons possible and indicate the effect of the degree of calcification.

4. Continuation of the Alkaline Earth Study

It is expected that by June all of the alkaline earths will have been compared on the basis of long term uptake, short term uptake, and short term removal of the alkaline earth metals. However, data on the effect of carrier upon the uptake and removal of these metals will still have to be obtained. Also, it would be of advantage to be able to compare these alkaline earths on the basis of the sites in bone where selection is occurring. By comparison it will be possible to determine if the selection is of the same nature as occurs with strontium.

For this bones will be labeled and after changing to a non-labeled medium will be removed and assayed at 10 and 30 minutes, 1, 2, 4, 6, 16, and 24 hours, and at 2, 4, 6, and 8 days. A removal curve would result that should make it possible to determine the number of compartments in which the isotope is dis-

tributed, their size, and their turnover times. Similar information would be obtained for uptakes by labeling bones for the periods of time stated above and plotting the uptake curves.

5. The Function of Enzymes in Selection

In testing live and heat killed bone it was determined that only live bone had a means of discrimination against strontium. To see if this is due to specific enzymes, known inhibitors such as beryllium, phlorizin, fluoroacetate, fluoride, and cyanide will be put into the medium. An inhibition of the selection mechanism will be looked for. The specific area in which the inhibition occurs will be determined by placing bone in labeled medium for 10 and 30 minutes, 1, 2, 4, 8, 16, and 24 hours. One-half of the bones will be placed in medium containing inhibitor and the other half will serve as controls. The strontium and calcium contents at each of these points will be determined and a curve plotted from the data. The equations for the curves should give the number, size, and mobility of the compartments. By comparing control and test groups an indication of the area of selection should be obtained. A similar experiment would be run for removal studies. Further investigation into the exact biochemical mechanisms will be made if the data appear promising.

6. Hormonal Effects

Bones will be labeled for periods of 24 hours, 2, 4, and 6 days and then transferred to a non-labeled medium containing parathyroid extract. Groups of the bones will then be removed at 30 minutes, 1, 2, 4, 8, 16, and 24 hours, and 2, 4, 6, and 8 days to attempt to ascertain the effect of the hormone upon the selection ratio and the removal of radiostrontium and radiocalcium from bone.

7. Effect of Temperature on Sr/Ca ratio in Bones

Data from short term uptake experiments indicated that selection against strontium for the shafts of bone occurred only at 39 degrees C. This would

tend to be evidence that a vital process is involved. To test this further, bones will be labeled for a period of 5 days and then removed to non-labeled medium. At this time equal sized groups will be placed in incubators at 4 degrees, 25 degrees, and 37 degrees Centigrade. The ratio of strontium to calcium will be determined at the end of a four day period. In addition, bones will be placed in labeled medium for several days to determine the Sr/Ca ratio under the same temperature conditions. If the experiments prove fruitful, an attempt will be made to determine the reason for the lack of selection.

Scientific Personnel

Principal Investigator: Lengemann, Frederick W., Ph. D., Assistant Professor of Chemistry. Thirty per cent of his time to be devoted to the project.

Information as to experience, training, and publications has been previously submitted in the original research proposal.

Other Personnel: A full-time technician, Mrs. Stuart Trapp, is to be employed and all of her time will be devoted to the project.

A part-time technician, all of his time to be spent on this project, will also be employed. It is intended to hire a medical or dental student for these purposes to perform the more tedious jobs and thus free skilled personnel for the more exacting details.

Other Financial Assistance

A proposed project is being submitted to the National Institutes of Health that will have as its major purpose the investigation of the effects of Vitamin D, parathyroid hormone, parotin, insulin, and thyroxine upon bone metabolism. It is intended to study the uptake of calcium and phosphorus simultaneously with the disappearance of accumulation of metabolites within the bone. None of the personnel to be employed or materials to be used for that research will be paid from

1108708

or purchased by funds provided by the Atomic Energy Committee.

Only twenty per cent of the senior investigator's time will be devoted to that project, thus continuing the thirty per cent allocation of his time to be spent on the A. E. C. contract.

All calculations of the University contribution to the A. E. C. research project have been made with the understanding that no project occupies the research facilities full time, Only that portion directly attributable to the A. E. C. has been taken as the University contribution.

Equipment and Facilities Available

The purchase of a scintillation counter, a large centrifuge, and a scaler during the past few months has materially increased the productiveness of my laboratory. An order has been placed, but not yet filled, to purchase a semi-automatic balance. The Department itself has recently acquired a Beckman DK-2, 2 recording spectrophotometers, and a Baird infra-red double beam spectrophotometer. Purchase of an ultra centrifuge is under way. These, in addition to the items of equipment and facilities listed in the original proposal, indicate that we are well equipped to carry out a research program from many angles of attack.

However, we have found that the major block in our research program is the voluminous amount of counting necessary. Especially during the time studies we produce large numbers of samples. Separating the bones into ends and shafts has doubled the amount of counting. To expedite this phase an automatic sample changer would be extremely valuable. Thus it should be possible to count samples almost continuously. Accuracy would be increased since we could then count for a given number of counts instead of the fixed time system now used. The present intention is to purchase an automatic sample changer and modify it so that it will count each sample twice, once without an absorber and once with the absorber.

Travel

The item of "travel" would be for the purpose of exchanging information, presenting papers, and to meet others interested in bone metabolism. It is planned to attend the Gordon Research Conferences and the Federation Meetings.

1108710

	<u>BUDGET</u>	
<u>Personnel</u>	<u>University</u>	<u>AEC</u>
Principal Investigator (30% of time)	\$1800.00	
Administrative Assistant	200.00	
Departmental Secretary	200.00	
Consultation (\$10.00 / hr.)		
Dr. Wood	400.00	
Dr. Williams	200.00	
Dr. Koeppe	300.00	
Dr. Cohn	200.00	
1-1/2 Technicians (includes 5-1/4% for retirement and social security)		\$4294.00
Part-time Secretary		100.00
 <u>Equipment</u>		
Automatic sample changer		1700.00
 <u>Supplies</u>		
Fertile eggs		400.00
Serum		300.00
Chicken plasma		50.00
Rats and feed		100.00
Radioisotopes		
Be-7		100.00
Ca-45 (Based on AEC discount		50.00
Sr-89 (50.00

1108711

BUDGET
(cont'd.)

<u>Supplies</u>	<u>University</u>	<u>AEC</u>
Special supplies (T.C. Detergent, G. M. Tube, Gloves, planchets, bacteriological pipettes, note books, data sheets, disposal of radioactive waste)		\$300.00
Common laboratory solvents and reagents	100.00	
Common laboratory glassware	150.00	
 <u>Travel</u>		 400.00
<u>Publication and Communications</u>		100.00
<u>*Utilities</u>	942.00	
<u>*Maintenance</u>	1081.00	
<u>*Library</u>	752.00	
<u>*Depreciation</u>	540.00	
*Loss of income	891.00	
 <u>SUBTOTAL</u>	 7756.00	 7944.00
Overhead (8%)		636.00
 <u>TOTAL</u>	 7756.00	 8580.00

Requested of A. E. C.

8580.00

*See additional sheets for computation of these costs.

1108712

Calculations of Indirect Costs

- Schedule I - Personnel supplied by Grant
- Schedule II- Equipment supplied by Grant
- Schedule III- Supplies included in Grant
- Schedule IV- Travel included in Grant
- Schedule V- Institutional personnel
(Detailed in budget)
- Schedule VI - Square footage of building space occupied part time
(But computed as full time occupancy by the Project)

Office and Laboratory	740 sq. ft.
Walk-in incubator	36
Sterilizing room	100
Shop	20
Cold room	30
Freezer room	14
Bulk storage room	20
Rat room	35
Dog and operating rooms	60
Constant temperature room	30
Dark room	9
High level isotope laboratory	
Division office	50
Adm. School Biological Sciences	90
Institutional Administration	221
Library	172
	<hr/>
	1637
"Dead Space" (total x 0.1)	164
	<hr/>
TOTAL	1801 sq. ft.

- Schedule VII- Cost of Utilities required but not included in Grant

Square footage (Schedule VI) x cost of heat, light, gas
and water/sq. ft.

1801 x \$0.523 = \$942.00

Schedule VIII - Cost for Maintenance and Custodial Service

Square footage (Schedule VI) x cost of custodial and
 maintenance / sq. ft. 1801 x \$0.60 = \$1081

Schedule X - Cost of Library x sum of Schedules I, II, III, and IV
Institutional operating expense
 $\frac{\$15,750}{167,104} \times \$8000 = \$ 752$

Schedule XI - Depreciation on Building Space and Equipment

Square footage (Schedule VI) x net building cost
 / sq. ft. x 0.02 = allowance for depreciation
 on construction.

1801 x \$ 11.25 x 0.02 = \$405

Square footage (Schedule VI) x equipment cost/sq. ft.
 x 0.05 = allowance for depreciation on equipment

1801 x 1.50 x 0.05 = \$315

TOTAL \$540

Schedule XII- Allowance for loss of income for expending funds for
constructing and equipping building space occupied
by the project.

Total capital outlay x 0.03 = loss of income

\$29,716 x 0.03 = \$891

SUMMARYExpenditures Required from the Granting Agency for the Project

1. Schedule I -	\$4394.00
2. Schedule II -	1700.00
3. Schedule III -	1450.00
4. Schedule IV -	400.00
	Subtotal
	7944.00
5. 8 % of Subtotal (Overhead)	<u>634.00</u>
	Total
	8578.00

Expenses Representing Institutional Contribution to the Project
and not included in the Grant

1. University contribution to consumable supplies	250.00
2. Schedule V	3300.00
3. Schedule VII	942.00
4. Schedule VIII	1081.00
5. Schedule X	752.00
6. Schedule XI	540.00
7. Schedule XII	<u>891.00</u>
	TOTAL
	\$7756.00

1108715

Expenditure Statement

Contract number AT-(40-1)-1829

Period: June 1, 1957 through Jan. 31, 1958

<u>Expenditures</u>	<u>AEC</u>	<u>University of Tennessee</u>
Personnel	1,538.50	
Retirement	154.76	
Supplies	1,178.89	
Equipment	2,097.81	
Travel	214.20	
Overhead	640.00	5,064.00
	<u>5,824.16</u>	<u>5,064.00</u>
Estimated expenditures to May 31, 1958	2,815.84	2,532.00
	<u> </u>	<u> </u>
Grand total	8,640.00	7,596.00

Funds available for the future financing include the contribution of \$7,756.00 by the University of Tennessee and that which the A.E.C. contributes to carry on this project.

Submitted March 1, 1958

O. W. Hyman

O. W. Hyman, Vice President
in Charge of Medical Units

1108716

Incident Report

No incidents occurred.

Radioisotope Purchase Report

Radioisotopes purchased or to be purchased at discount include:

<u>Isotope</u>	<u>AEC Price</u>	<u>Price paid</u>	<u>Savings</u>
Ba-133P	\$100.00	\$20.00	\$80.00
Sr-89	10.00	2.00	8.00
			<hr/>
		Total	\$88.00

1108717

TO : Addressees Listed Below

DATE: May 9, 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.

Contract AT-(40-1)-1829 with the University of Tennessee

<u>Addressees:</u>	<u>Division:</u>	<u>Init:</u>	<u>Date:</u>	<u>Remarks:</u>
1. RALPH ELSEN	Contract	5/20/57	W.E.	
2. A. E. Miller	Budget	W.E.	5-13	06-71-91 (24) Funds
3. C. S. Shoup	Research & Development	W.E.	5/14	
4. L. D. Mackay	Finance	W.E.		
5.				
6.				

RETURN TO:

Alice Brown

Contract Division

Telephone: 4719

1108718

Office Memorandum • UNITED STATES GOVERNMENT

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

DATE: May 3, 1957

FROM : John R. Moore, Director, Contract Division

SUBJECT: REQUEST FOR PREPARATION OF CONTRACT WITH THE UNIVERSITY OF TENNESSEE

SYMBOL: ACC:BSH

Please prepare a new contract with the University of Tennessee for a period of one year beginning June 1, 1957, with Commission funds in the amount of \$8,640. Also designate Dr. Frederick W. Lengemann as Senior Investigator and include provisions for compliance with AEC Manual Chapter 7510.

There is enclosed herewith a Request for Contract Action dated April 30, 1957 from C. S. Shoup, Research and Development Division.



John R. Moore

Enclosures:

1. Request for Cont. Action
2. Budget
3. Resume'
4. Cont. Authorization
5. Notification of cont. negotiation
6. Proposal

CC: L.D. MacKay
C. S. Shoup
A. Brown, w/encls. 1, 2 & 4

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

7. Request Submitted By: (signed) A. Schuler

Date APR 30 1957 Title: see S. Shoup

C. S. SHOUP
CHIEF, BIOLOGY BRANCH
RESEARCH AND DEVELOPMENT DIVISION

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

Headquarters designated research contract
TITLE: "The Metabolism of Alkaline Earth Metals by Bone"

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

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

New contract for a period of one year beginning ^{June} ~~May~~ 1, 1957, with
Commission funds in the amount of \$8,640. Designate Dr. Frederick W.
Lengemann as Senior Investigator. Include provisions for compliance
with AEC Manual Chapter 7510.

(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)

Memorandum from Charles W. Shilling to S. A. Sapiris, dated April 17, 1957.

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

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

12. Disputes:

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

None

1108720

1. Chairman
TO: J. B. Moore Contract B. No. From: Res. B. Act. 1957

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.

The University of Tennessee
Memphis, Tennessee

Review and approval of Contract, Subcontract or Purchase Order.

Number: _____

Name: _____

Modification of Contract

No. _____

Contractor: _____

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 \$ 3,640.00

Source of Funds

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

Project No. _____ or, Activity No. 6220

Funds to be Obligated: Allotment No. 06-74-91(24) (F.Y. 1957 Funds)

Procurement Directive No. BM-57-293 Dated 4-17-57

Issuing Office Division of Biology & Medicine

Concurrence in Funding Statement: (signed) _____

A. 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:)

AGREEMENT FOR NEW CONTRACT - DR. F. W. LINGEMANN
PERIOD 6-1-57 - 5-31-58

1) <u>Salaries and Wages:</u>		\$6,390.00
Dr. F. W. Lingemann (30% of time)	\$1,690.00	
Consultants	1,100.00	
Research technician	3,150.00	
Administrative and Secretarial Assistance	450.00	
2) <u>Equipment:</u>		2,100.00
3) <u>Supplies:</u>		2,500.00
4) <u>Publication and Communications:</u>		100.00
5) <u>Travel:</u>		300.00
6) <u>Overhead and Indirect Costs:</u>		<u>4,846.00</u>
	Total	\$16,236.00

The Commission's contribution to the above budget will be \$5,000.

1108722

UNIVERSITY OF TENNESSEE
COLLEGE OF MEDICINE
DEPARTMENT OF CHEMISTRY
MEMPHIS, TENNESSEE

Dr. Frederick T. Lengenann
Project Leader.

THE METABOLISM OF ALKALINE EARTH METALS BY BONE

The contractor will undertake studies on the metabolism of alkaline earth metals in bone, comparing the metabolism of the various members of the alkaline earth group of elements, with reference to bone deposition, atomic structure, chemical properties of the minerals and bone materials, etc. Phases of the mineral metabolism will be investigated in growing bone to determine processes under enzyme influence, the rate of strontium calcium removal from bone and means to alter it, and the mechanism by which stable strontium inhibits calcification and increases discrimination against radioactive strontium by bone. Investigations will be made into initial stages of calcium-strontium uptake by bone, and into long-term uptake, turnover, and removal of strontium. Various associated work will involve further elucidation of the means by which strontium and calcium are mutually involved in the long-term metabolism of important animals.

~~Contract approved, April 17, 1957 at \$8640 of AEC money on a t. c. of \$16,236.00.~~

C. S. St. ...

1108723

UNITED STATES ATOMIC ENERGY COMMISSION
WASHINGTON, D. C.

Contract Authorization No. EM-57-293

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

APR 17 1957

FROM : Charles 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:JFB**

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: **University of Tennessee**
2. Investigator (s): **Frederick W. Lengemann, Ph.D.**
3. Title: **"The Metabolism of Alkaline Earth Metals by Bone"**
4. () New Contract, () Renewal of Contract No. _____
5. Duration: **one year**
6. AEC Technical Representative: **John F. Bonner** *JFB*
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>
<u>06-71-91 (24)</u>	<u>6220</u>	<u> </u>	<u>\$ 8,640</u>	<u>\$ 8,640</u>
<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>

APR 18 1957

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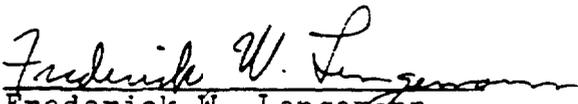
1108724

A PROPOSAL TO
The Atomic Energy Commission
Division of Biology and Medicine

For a Grant to Provide Funds for a Project Entitled
"The Metabolism Of Alkaline Earth Metals by Bone"

Division of Chemistry
School of Biological Sciences
University of Tennessee
Memphis

Principal Investigator


Frederick W. Lengemann,
Instructor in Chemistry

Date: June 18, 1956


O. W. Hyman, Vice-President
in Charge of Medical Units

110872b

Project Proposal to be Submitted to the A.E.C.

Title: The Metabolism of Alkaline Earth Metals by Bone

Institution: Division of Chemistry
School of Biological Sciences
University of Tennessee
Memphis, Tennessee

Scientific Background

The metabolism of strontium has been of interest for many years. Because of its position in the periodic table and its ability to be deposited in bone, it has always been compared to calcium. Early workers (Shipley et al., 1922; Kinney and McCollum, 1923) conclusively demonstrated that strontium could not completely replace calcium as the structural element of bone. However, during the study of the metabolism of strontium-90, workers persistently stressed the similarities. Comar, Whitney, and Lengemann (1953) reiterated that the metabolic patterns differed by showing that dietary calcium was preferred over dietary strontium-90 by a factor of 3.6 to 1.

This finding stimulated research to find the site of the selection against strontium. MacDonald and co-workers (1956), Bauer, et al. (1956), and Comar et al. (1956) found that in animals the intestinal tract as well as the kidney contributed to the overall selection against strontium. In these experiments, bone did not appear to have a discernable preference for either calcium or strontium. However, when bones were grown in the presence of constant levels of calcium-45 and strontium-89 in an in vitro culture, Lengemann (1956) was able to demonstrate that bone did show an overall selection against strontium. This selection took place with carrier-free radiostrontium and also when 0.1 mg per cent carrier strontium was added to the medium.

1108727

Further investigation revealed that 2 hours after exposure to the labeled medium 1.08 times more strontium than calcium was present in the bones. Nevertheless, 7 days later the Sr*/Ca* ration had equilibrated at 0.83. By replacing the labeled with non-labeled medium it was possible to determine that about 1.2 times as much strontium as calcium was leaving bone.

The observation that strontium enters bone in a somewhat greater ratio than calcium has confirmation. MacDonald et al. (1956) reported that 2 hours after an intraperitoneal dose the Sr*/Ca* ratio in the femurs of rats was 1.1. Van Cleeve (1956) also found more strontium than calcium in rat femur shafts 15 minutes after intraperitoneal dosage. Bauer et al. (1956) reported that barium too appeared to enter bone at a faster rate than calcium.

That a Sr*/Ca* ratio greater than 1 was found in bone would question that exchange was the principal mechanism for the entry of radioisotopes into growing bone since MacDonald et al. (1956) and Whitney and Comar (1952) have shown that radiostrontium and radiocalcium exchange into powdered bone in a 1:1 ratio. The evidence would favor the idea, as expounded by Bauer et al. (1954), that the incorporation of radioisotopes in growing bone comes about through the process of accretion of new bone salt.

A strontium-calcium ratio greater than 1 would also tend to rule out the tertiary and secondary phosphates of calcium as being the salt initially precipitated from blood since the strontium salts are more soluble than those of calcium (Holt et al., 1954). The same results were found for the secondary and tertiary phosphates of magnesium and barium except

that $BaHPO_4$ was more soluble than the corresponding calcium salt. The character of the initial precipitate could receive some elucidation if a comparison was made of the amount and the ratio of entry into powdered and growing bone of the elements of the second group of the periodic table.

That more strontium than calcium left bone (Lengemann, 1956) was a new observation that subsequently was verified by Talmage and Comar (1956) and shown to occur in vivo. This selection could possibly account for the data of Norris and Kisielewski (1948) where it was noted that 10 days after deposition radiostrontium appeared to leave bone at a faster rate than calcium. It is interesting that the metabolic behavior of radium did not differ appreciably from that of calcium.

To explain the greater loss of strontium than calcium from bone is difficult. As mentioned before, the phosphate salts of strontium have been noted as being more soluble than those of calcium. The recrystallization process, as expounded by Neuman and Neuman (1953), would make it possible for a crystal to be repeatedly subjected to the selective processes and thus account for the bone ratio changing from 1.08 and 0.83. Solubility differences could be the mechanism involved but this not a necessity. MacDonald et al. (1952) noted that both strontium and lead are incorporated into bone crystal but that the crystal planes are distorted. This strain possibly could aid in the selection by slowing the process of crystal formation giving time for a calcium ion to exchange for that of strontium.

That strontium leaves bone in greater amounts than calcium could be an important fact if it was possible to accentuate the selection. Hamilton, Greenberg, and Copp (1946, 1947) have pursued the problem of the removal of fission products from bone but the results have not been encouraging. Their methods, as those of Ray et al. (1956), consisted for the most part of the same procedures used in cases of radium poisoning. Only methods that resulted in bone destruction seemed to increase the excretion of strontium that had been in bone more than 10 days.

The selective removal of strontium or other metals from bone can only be intelligently attempted if the processes involved can be elucidated. These processes may be purely physical-chemical or they may be under the control of enzymes. Enzymes have been shown to participate in the calcification process of in vitro cartilage slices (Gutman et al., 1942; Goldenberg and Sobel, 1952; Mark et al., 1953). That parathyroid hormone acts directly on bone (Talmage et al., 1953) to remove calcium suggests that other than physical-chemical properties are involved in this phase as well.

An estimate of the importance of enzyme systems and their contribution to the selective mechanisms of bone is susceptible to solution by the use of in vitro cultures of growing bone. Exploratory work in this laboratory has found that stable strontium is a potent inhibitor of calcification in growing bone. This is in agreement with in vitro and in vivo observations (Shorr and Carter, 1952; Sobel et al., 1951). In addition, the presence of small amounts (0.1 mg %) of stable strontium in the medium tended to increase the selection against strontium-89 by bone.

Bibliography

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Talmage, R.V., Lotz, W.E., and Comar, C.L. (1953) Proc. Soc. Expt. Biol. and Med. 84, 578.

VanCleeve, (1956) Unpublished Data.

Whitney, I.B., and Comar, C.L., (1952) Prog. Rpt. U.T.-A.E.C.

Scientific scope of the proposed research.

The major objectives are:

- A. A comparison of the metabolism by bone of the members of the alkaline earth group. This would provide data on the deposition of important bone-seeking radioelements and make it possible to relate atomic structure and known chemical properties to suitability as a bone mineral.
- B. Investigation of the phases of mineral metabolism of growing bone to determine what processes are under the influence of enzymes.
- C. To determine the rate of strontium and calcium removal from bone and by various means to attempt to alter it.
- D. To describe the mechanism by which stable strontium inhibits calcification and increases the discrimination against radioactive strontium by bone.

It is unknown at this time whether other laboratories are engaged in studying the metabolism of calcium and strontium with these same objectives in mind. However, it should be pointed out that the in vitro bone culturing technique, as used in this laboratory, is the most direct approach now possible. The demonstration that strontium is not metabolized in bone as is calcium points up the superiority of this method. Thus, it is felt that it will be possible to make contributions not attainable by more orthodox procedures.

Experimental Plan

The in vitro procedure to be used in this study will basically be that used in tissue culture. Whole femurs and tibias from 7-day old chick embryos will be adhered to the walls of a test tube by coating the walls with plasma, positioning the bones, and then causing the plasma to clot by adding a few drops of embryo extract. The nutrient medium will be added and the tubes placed in a roller-tube apparatus and incubated. The medium will be changed every 2 or 3 days. Using this technique the following investigations will be carried out:

1. Comparative Metabolism of the Alkaline Earths by Bone

The uptake of Ca-45 by bone will be used as the standard and, in double tracer experiments, will be compared to that of Be-7, Sr-39, Ba-140, Ra and, if arrangements can be made, Mg-28. The ratio of entry into bone (Ca-45 = 1) will be determined by allowing live and powdered bone to come in contact with the radioisotopes for periods of time varied from 10 minutes to several hours. The equilibrium ratio will be found by growing the bone for several weeks in the presence of constant levels of the radioactivity. Removing some of these doubly labeled bones to a radioisotope-free medium it will be possible to obtain the removal ratio for the alkaline earth metal being compared to calcium. Accretion and resorption rates can then be calculated and used for comparative purposes.

By growing the embryonic bones in the presence of graded levels of the different alkaline earth metals (where possible) the toxicity of the metal for calcification and growth will be determined, as will the effect of the element upon the selection mechanisms of bone.

2. Comparative Strontium and Calcium Metabolism of Bones as Affected by the Type of Serum, Age of Bone, and Species.

The procedures used in Part 1 will also be used in this experiment to determine the initial, equilibrium, and removal ratios. The first phase would be to see if the type of serum used as the medium has an effect upon the ratios. For this embryonic bone will be grown in the sera from chickens, dogs, cattle, sheep, and man. Should any differences be encountered, the distributions of Ca-45 and Sr-89 in the sera will be investigated.

The second phase will be to compare calcium and strontium metabolism in embryonic and postnatal bone from chickens, rats, mice, and if specimens can be obtained, man.

3. Investigations into the Initial Uptake of Calcium and Strontium

A. To determine the time required to change the initial 1.08 ratio to the 0.83 ratio at equilibrium, bone will be placed in the doubly labeled medium for 15, 30, and 60 minutes, and 2, 3, 4, 6, 8, and 24 and then the Sr*/Ca* ratio will be determined. This should also give an estimate of the ratio at "0" time.

B. The Sr*/Ca* ratio after a 2 hour exposure to the radioisotopes will be determined in live bone and will be compared to that of powdered bone, and bone that has been killed by heat. Should the results indicate that enzyme systems are involved in this phase of bone mineralization then known enzyme inhibitors such as Be, phlorizin, CN^- , F^- , and fluoroacetate will be added to the medium to try to prevent the uptake of the calcium and strontium.

C. The rate of removal of the radioisotopes deposited in live, powdered, and killed bones will be estimated by removing the labeled material to a non-labeled medium. This should estimate the ratio of removal as applied to the material initially deposited in bone and indicate whether or not a solubility phenomenon is operating.

D. Several levels of stable strontium will be added to the medium to try to inhibit the initial uptake of calcium. The stable strontium will be added with Ca-45 and Sr-89 in one experiment and in another will precede the radioactivity by several days. After 2 hours of exposure some of the bones will be assayed for radioactivity and the rest will be placed on non-labeled medium to measure the rate of removal.

4. Long Term Uptake, Turnover, and Removal of Calcium and Strontium

A. To determine if the ratio of removal is a constant or is variable, bone will be grown for a 7-day period in the presence of Sr-89 and Ca-45. Some of the bones will then be assayed for Ca-45 and Sr-89 and the rest will be transferred to non-labeled medium. These bones will be assayed at 1, 2, 4, 6, and 8 days after the transfer.

B. If enzymes are involved in the removal of Sr-89 and Ca-45 from bone then by putting labeled bones that have been killed by heating into a non-labeled medium a different removal ratio than that of live bone should be observed. Should enzymes be involved then inhibitors will be added to the systems to try to stop or alter the removal of radionuclides.

C. The effect of stable strontium on the ratio and amount of removal of Ca-45 and Sr-89 will be estimated. Labeled bone that has been grown

in the presence of stable strontium for the entire growth period will be compared to bone to which stable strontium will be added at the time of transfer to the non-radioactive medium. Estimates of radioactivity will be made at 1 and 5 days.

5. Preliminary Experimentation

This phase will consist of short experiments to determine the effect of various hormones upon strontium and calcium metabolism of bone grown in vitro.

Scientific Personnel

Principal Investigator: Lengemann, Frederick W.; Ph.D., [REDACTED]

[REDACTED]. Instructor in the Division of Chemistry. Thirty per cent of time to be devoted to project.

Experience - 1948-1951

Parttime laboratory assistant while at Cornell University. Was employed on a project studying the sodium chloride requirements of dair cattle.

1951-1953

Worked on thesis problem for the Ph.D. degree at the [REDACTED]. The problem involved counting and culturing of rumen bacteria, counting of protozoa, determination of the lower fatty acids and the "B" vitamin levels in the rumen contents of young dairy calves.

1954-55

Employed at the U.T.-A.E.C. AGR. RES. PROG. at Oak Ridge as a Research Associate. The research involved the study of endogenous calcium and phosphorus in cattle, toxicity of radiochromium and radiozinc in rats,

comparative metabolism of radiostrontium and radiocalcium in cattle and rats, comparison of radiostrontium and radium in sheep, and the metabolism of I-131 by dairy cattle.

July 1955 to present:

Instructor in Chemistry at the University of Tennessee at Memphis. A laboratory has been outfitted and a technique of growing bone in vitro developed. This method has been successfully employed with studies of calcium and strontium metabolism.

Summer of 1956:

Summer Participant at the Oak Ridge Institute of Nuclear Studies, Medical Division. The studies consisted of determining the the absorption of calcium from milk, strontium-85 absorption in humans, the transfer of calcium across the intestinal wall, the absorption radioiron and radionickel, and the comparative metabolism of calcium and strontium in lactating goats.

Publications

1. THE NORMAL CHLORIDE CONCENTRATION OF BLOOD PLASMA, MILK, AND URINE OF DAIRY COWS
F. W. Lengemann, P. D. Aines, and S. E. Smith Cornell Vet. 42;
1 - 9 (1952)
2. BLOCK VS. LOOSE SALT CONSUMPTION BY DAIRY CATTLE
S. E. Smith, F. W. Lengemann and J. T. Reid J. Dairy Sci. 34;
762 - 765 (1953)
3. THE DEVELOPMENT OF RUMEN FUNCTION IN THE DAIRY CALF
F. W. Lengemann Thesis Univ. of Wis. (1953)
4. THE DEVELOPMENT OF RUMEN FUNCTION IN THE DAIRY CALF I. SOME CHARACTERISTICS OF THE RUMEN CONTENTS OF CATTLE OF VARIOUS AGES
F. W. Lengemann, and N. N. Allen J. Dairy Sci. 38; 651 - 656 (1955)
5. COMPARATIVE UTILIZATION OF SR-90 AND CALCIUM BY DEVELOPING RAT FETUS AND THE GROWING RAT
C. L. Comar, I. B. Whitney, and F. W. Lengemann Proc. Soc. Expt. Biol. and Med. 88; 232 - 236 (1955)

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Publications (contd.)

6. THE SECRETION OF I-131 IN MILK
F. W. Lengemann, R. A. Monroe, and E. W. Swanson J. Dairy Sci.
38; 619 (1955)
7. THE SECRETION OF THE MINERALS OF MILK AS STUDIED WITH RADIOISOTOPES
F. W. Lengemann and C. L. Comar Conf. on Radioactive Isotopes
in Agric. (Jan. 1956)

Submitted for Publication:

8. THE COMPARATIVE METABOLISM OF SR^{89} and CA^{45} BY BONE GROWN IN VITRO
F. W. Lengemann Proc. Soc. Expt. Biol. and Med. (in press)
9. ABSORPTION OF CALCIUM AND STRONTIUM FROM MILK AND NON-MILK DIETS
F. W. Lengemann, C. L. Comar, and R. H. Wasserman J. Nutr.
(submitted for publication)
10. THE EFFECT OF SEASON ON THE SECRETION OF IODINE IN MILK
F. W. Lengemann, F. W. Swanson, and R. A. Monroe J. Dairy Sci.
(submitted for publication)
11. SECRETION OF IODINE IN MILK OF DAIRY COWS USING DAILY ORAL DOSES OF
 I^{131}
F. W. Lengemann, and F. W. Swanson J. Dairy Sci.
(submitted for publication)

Other Personnel

Fulltime Technician - all of time on project

Other Financial Assistance

None

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Equipment and Facilities Available

Those items available for this project consist of rollertube apparatus, drying ovens, pH meter, analytical balance, survey meter, microtome, microscope, embedding oven, autoclave, radioisotope fume hood, dishwasher, sterilizing oven, and a clinical centrifuge. The laboratory is well furnished and in addition there is a walk-in refrigerator, walk-in incubator, dog room, rat rooms, dark rooms, and a high level radioisotope laboratory.

For this research a scintillation counter and a large centrifuge would need to be provided. Since many of the isotopes planned to be used in this project are gamma emitters a scintillation counter is a necessity. There is none available for this project. The centrifuge is needed in the preparation of blood serum for medium, in the production of the chick embryo extract, and for determinations of stable calcium. The level of activity planned for this project would make it a necessity.

Travel and Other Items

The item of "Travel" would be for the purpose of exchanging information, presenting papers, and to meet others interested in bone metabolism. It is planned to attend the Gordon Research Conferences and the Federation Meetings.

BUDGET

<u>Personnel</u>	<u>University</u>	<u>AEC</u>
Principal Investigator (30% of time)	1690.00	
Administrative Assist.	150.00	
Departmental Secretary	200.00	
Consultation (\$10.00 / hr.)		
Dr. Wood	400.00	
Dr. Williams	200.00	
Dr. Perkinson	300.00	
Dr. Cohn	200.00	
Technician (includes 5% retirement)		3150.00
Parttime Secretary		100.00
 <u>Equipment</u>		
Scintillation Counter		1000.00
Centrifuge (SB-2) and Accessories		1100.00
 <u>Supplies</u>		
Fertile Eggs		400.00
Serum		300.00
Chicken Plasma		50.00
Rats and Feed		100.00
Radioisotopes		
Mg-28		500.00
Ra		200.00
Be-7		100.00
Ca-45		50.00
Sr-89		50.00
Ba-133		200.00

BUDGET
(Contd.)

<u>Personnel</u>	<u>University</u>	<u>AEC</u>
Special Supplies (T.C. Detergent, G. M. Tube, Gloves, Planchets, Bacteriological P. Pettes, Note Books, Data Sheets, etc.)		300.00
Common Laboratory Solvents and Reagents	100.00	
Common Laboratory Glassware	150.00	
<u>Travel</u>		300.00
<u>Publication and Communications</u>		100.00
<u>*Utilities</u>	942.00	
<u>*Maintainance</u>	1081.00	
<u>*Library</u>	752.00	
<u>*Depreciation</u>	540.00	
<u>*Loss of Income</u>	891.00	
<u>SUBTOTAL</u>	7596.00	8000.00
Overhead (8%)		640.00
<u>TOTAL</u>	7596.00	8640.00

Requested of A. E. C. \$8640.00

* See additional sheets for computation of these costs.

Calculations of Indirect Costs

- Schedule I - Personnel Supplied by Grant
Schedule II - Equipment supplied by Grant
Schedule III - Supplies included in Grant
Schedule IV- Travel included in Grant
Schedule V- Institutional Personnel
(Detailed in Budget)
Schedule VI - Square Footage of Building Space occupied parttime.
(But computed as fulltime occupancy by the Project).

Office and Laboratory	740 sq. ft.
Walk-in incubator	36
Sterilizing room	100
Shop	20
Cold room	30
Freezer room	14
Bulk storage room	20
Rat room	35
Dog and operating room	60
Constant temperature room	30
Dark room	9
High level isotope laboratory	20
Division office	50
Adm. School Biological Sciences	90
Institutional Administration	221
Library	<u>172</u>
	1637
"Dead Space" (total x 0.1)	<u>164</u>
TOTAL	1801 sq. ft.

Schedule VII - Cost of Utilities Required But not Included in Grant

Square Footage (Schedule VI) x cost of heat, light, gas, and
water/sq. ft.

$$1801 \times \$0.523 = \$942.00$$

Schedule VIII - Cost for Maintenance and Custodial Service

Square footage (Schedule VI) x cost of custodial and maintenance/sq.ft.

$$1801 \times \$0.60 = \$1081$$

Schedule X - Cost of Library

Operating expense of library x sum of schedules I, II, III, and IV
Institutional operating expense

$$\frac{\$15,750}{167,104} \times \$8000 = \$752$$

Schedule XI - Depreciation on Building Space and Equipment

Square footage (Schedule VI) x net building cost/sq. ft. x 0.02 =
allowance for depreciation on construction

$$1801 \times \$11.25 \times 0.02 = \$405$$

Square footage (Schedule VI) x equipment cost/sq. ft. x 0.05 =
allowance for depreciation on equipment

$$1801 \times 1.50 \times 0.05 = \$315$$

TOTAL \$540

Schedule XII - Allowance for loss of income for expending funds for
constructing and equipping building space occupied by the project.

Total capital outlay x 0.03 = loss of income

$$\$29.716 \times 0.03 = \$891$$

SUMMARY

Expenditures Required From the Granting Agency For the Project

1. Schedule I -	3250.00
2. Schedule II -	2100.00
3. Schedule III -	2350.00
4. Schedule IV -	300.00
Subtotal	8000.00
5. 8 % of Subtotal (Overhead)	640.00
Total	8640.00

Expenses Representing Institutional Contribution to the Project
and not Included in the Grant

1. University contribution to consumable supplies	250.00
2. Schedule V	3140.00
3. Schedule VII	942.00
4. Schedule VIII	1081.00
5. Schedule X	752.00
6. Schedule XI	540.00
7. Schedule XII	<u>891.00</u>
TOTAL	\$7596.00