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Dr. William G. Pollard
Executive Director
Oak Ridge Associated
Universities
Post Office Box 117
Oak Ridge, Tennessee

REPOSITORY Oak Ridge Operations
Records Holding Area
COLLECTION Documents 1944-1984
BOX No. H-66-4 Bldg 2714-H
Lipid Markers in Human
FOLDER Leukemia Cells # 20-10-74

Dear Dr. Pollard:

ORAU GRANT ENTITLED "LIPID MARKERS IN HUMAN LEUKEMIA CELLS"
(AEC GRANT NO. 20-10-74)

Your letter of June 21, 1974, informed us that the Corporation has received a one-year grant commencing July 1, 1974, from the Leukemia Research Foundation, Inc., in the amount of \$13,500 to support a portion of the direct and indirect costs related to the subject project. The project will be under the direction of Dr. Fred L. Snyder.

The AEC has determined a programmatic interest in the subject research project and is willing to jointly support the project through July 1, 1975, by providing the use of AEC facilities and equipment, approximately 5% of Dr. Snyder's time, and a portion of the applicable indirect costs (difference between 8% of TDC and the established ORAU rate). The AEC's approval of this joint support is made subject to the arrangements outlined in the proposal dated February 11, 1974, and your letter of June 21, 1974.

Sincerely,

ORIGINAL SIGNED BY
RICHARD L. EGLI, Acting

Joseph A. Lenhard, Director
Research and Technical Support Division

ORR:JDB

- Enclosures: *1077 L*
1. ORAU ltr dtd 6-21-74
2. Leukemia Research ltr dtd 6-14-74

- cc w/encl:
J. L. Liverman, HQ
J. H. Hill
C. W. Hill
T. W. White, Jr. (2)
W. H. Henderson

T 777

ORGANIZATION & MANAGEMENT - 12-2
60-10-74

OFFICE ▶	R&D Branch Burlinson	R&TS Div.	
SURNAME ▶	<i>[Signature]</i>	<i>[Signature]</i>	
DATE ▶	6-25-74	JUN 27 1974	

1036348

Oak Ridge Associated Universities

Incorporated

P.O. Box 117 · Oak Ridge, Tennessee 37830

June 21, 1974

Telephone 615 483-8411

Mr. Joseph A. Lenhard, Director
Research and Technical Support Division
U. S. Atomic Energy Commission
Oak Ridge, Tennessee 37830

Subject: NOTICE OF RECEIPT OF GRANT FROM LEUKEMIA RESEARCH
FOUNDATION - JUNE 14, 1974

Dear Mr. Lenhard:

We have received a grant from the Leukemia Research Foundation for \$13,500 in support of a research project in "Lipid Markers in Human Leukemic Cells" for the period July 1, 1974 to July 1, 1975. The proposal was sent to your office on February 11, 1974.

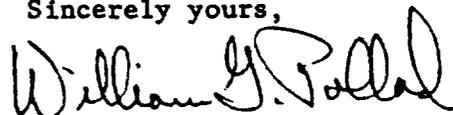
The work will be carried out in AEC facilities and some AEC equipment will be used on the project. Dr. Fred Snyder will provide technical guidance to the project of up to 5% of his time but no charge will be made to the grant. All other assigned personnel will be corporate employees.

The grant provides maximum overhead of 8% of direct costs. It is requested that the difference in the amount of overhead costs provided by the grant and the actual costs be borne by the AEC Medical Division FY 1975 budget. This amount is approximately \$3,600.

Your joint support of this project is requested as part of the established lipids program.

Copy of the grant award is enclosed.

Sincerely yours,



William G. Pollard
Executive Director

T 7/52;

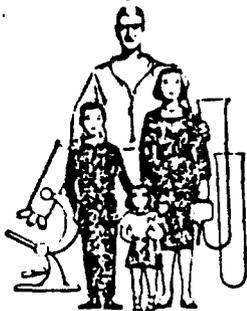
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ORGANIZATION & MANAGEMENT - 12-2 (E R 24)

20-10-74



LEUKEMIA

research foundation, inc.

333 N. Michigan Ave. Chicago, Illinois 60601 FRanklin 2-2186

COUNCIL (LEUKEMIA RESEARCH FOUNDATION)

June 14, 1974

Dr. Fred L. Snyder
Head, Biological Chemistry
Oak Ridge Associated Universities
P. O. Box 117
Oak Ridge, Tennessee 37830

Dear Dr. Snyder:

Thank you for your letter of June 5th. I am happy to report that funding for your project has been approved.

Please refer to my letter of May 28th for details about presentation of your check. We will appreciate hearing from you just as soon as possible and hope to have the pleasure of seeing you this coming week end.

Sincerely yours,

LEUKEMIA RESEARCH FOUNDATION, INC.

SEYMOUR L. KRAMER, Chairman,
Medical Advisory Board Committee

Medical Advisory Board

Dr. Howard L. Alt
Dr. Eric Brown
Dr. Israel Davidsohn
Dr. Paul Hettler
Dr. Leon Jacobson
Dr. Aaron M. Josephson
Dr. Stephen U. Schwartz
Dr. Irving Shulman
Dr. Paul Szanto
Dr. Stanley Yachnin

SLK:s

cc: Mr. W. C. Pollard

Honorary Board

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OAK RIDGE ASSOCIATED UNIVERSITIES

INCORPORATED

P. O. BOX 117

OAK RIDGE, TENNESSEE 37830

February 11, 1974

AREA CODE 615
TELEPHONE 483-6411

*HE approved -
not signed yet - 1-31-74
AB*

Mr. Joseph A. Lenhard, Director
Research and Technical Support Division
U. S. Atomic Energy Commission
Oak Ridge, Tennessee 37830

Subject: RESEARCH PROPOSAL ENTITLED "LIPID MARKERS IN HUMAN LEUKEMIC CELLS"

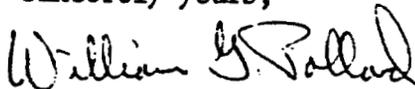
Dear Mr. Lenhard:

20-10-74

Enclosed are three copies of the above referenced research proposal which we are forwarding to the Leukemia Research Foundation, Inc., Chicago, Illinois. A draft copy of this proposal was forwarded to your office for review on January 16, 1974. Dr. Benson of your staff notified Mr. Crockett of the Medical Division that the proposal was approved and could be forwarded to the Leukemia Foundation.

The proposed research will be carried out in AEC facilities operated by the Oak Ridge Associated Universities' Medical Division. We shall keep you informed of the Foundation's action relative to this proposal and shall be prepared to consider with you any special arrangements the Commission may wish to make in order that the work may be carried out in AEC facilities.

Sincerely yours,



William G. Pollard
Executive Director

CROCKETT:wfl
Enclosures D1<

ADMINISTRATION & MANAGEMENT - 12-2(

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20-10-74

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FEB 15 1974

J. L. Liverman, Director, Division of Biomedical and Environmental Research, HQ

ORAU PROPOSAL FOR GRANT TO LEUKEMIA RESEARCH FOUNDATION, INC. (AEC NO. 20-10-74)

Pursuant to my memorandum of January 22, 1974, enclosed for your information is a copy of the formal proposal entitled "Lipid Markers in Human Leukemic Cells," submitted by the Oak Ridge Associated Universities to the Leukemia Research Foundation, Inc. (LRF)

In addition, this memorandum confirms your verbal approval given on January 31, 1974, for AEC joint support of the above ORAU project based on AEC's determination of programmatic interest in the proposed research.

ORIGINAL SIGNED BY:
RICHARD L. EGLI

for
Joseph A. Lenhard, Director
Research and Technical Support Division

ORR:JDB

Enclosure:
Proposal

OK
CC: C. W. Hill

T 1786

ORGANIZATION & MANAGEMENT -12-2 ORAU

OFFICE ▶	R&D Branch Burleson:nb	R&TS Div.				
SURNAME ▶	<i>Burleson</i>	<i>EL</i>			20-10-74	
DATE ▶	2-14-74	2/14/74				

1036352

OAK RIDGE ASSOCIATED UNIVERSITIES
INCORPORATED
P. O. BOX 117
OAK RIDGE, TENNESSEE 37830

AREA CODE 615
TELEPHONE 483-8411

February 11, 1974

Dr. Seymour L. Kramer
666 North Lake Shore Drive
Chicago, Illinois 60611

Subject: RESEARCH PROPOSAL ENTITLED "LIPID MARKERS IN
HUMAN LEUKEMIC CELLS"

Dear Dr. Kramer:

We are submitting for your consideration 14 copies of an application for support of a research project entitled "Lipid Markers in Human Leukemic Cells." The project would be supervised by Dr. Fred L. Snyder of our Medical Division staff.

Oak Ridge Associated Universities is a nonprofit corporation sponsored by 42 Southern universities. The major portion of its activities are carried out under a long-term operating contract with the U. S. Atomic Energy Commission. This proposed research would be carried out in AEC-owned facilities. These facilities, insofar as they may be required for work under this proposal, may be used only as the AEC may approve. Our AEC contract contemplates the possibility of our performing such work under its terms as may be agreed upon between AEC and the Leukemia Research Foundation and no charge would be made for use of government-owned facilities.

If questions arise during the review of this proposal, please do not hesitate to call Dr. Fred Snyder (AC 615 483-8411, extension 291).

Sincerely yours,

Original signed by
William G. Pollard

William G. Pollard
Executive Director

CROCKETT:ahp
Enclosures

bc: Mr. Lenhard, AEC/ORO
Executive Office

Mr. Rose Dr. Andrews Dr. Snyder Mr. Crockett

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COUNCIL (LEUKEMIA RESEARCH FOUNDATION)

APPLICATION FOR A GRANT IN LEUKEMIA RESEARCH
(14 COPIES TO BE SUBMITTED.)

DATE: 11 February 1974

DATE PROJECT IS TO BEGIN: July 1974

1. TITLE OF PROJECT:

"Lipid Markers in Human Leukemic Cells"

2. NAME OF PRINCIPAL INVESTIGATOR: Fred L. Snyder

DEGREE: Ph. D.

OFFICIAL POSITION AND DEPARTMENT: Chief Scientist
Biological Chemistry

MAILING ADDRESS: Medical Division
Oak Ridge Associated Universities
P. O. Box 117; Oak Ridge, Tennessee 37830

3. NAME OF APPLICANT'S ORGANIZATION:

Oak Ridge Associated Universities

ADDRESS: P. O. Box 117
Oak Ridge, Tennessee 37830

4. NAME AND TITLE OF OFFICIAL RESPONSIBLE FOR ADMINISTRATION OF RESEARCH FUNDS:

William G. Pollard
Executive Director

5. DOES RESEARCH INVOLVE HUMAN SUBJECTS? YES: X NO
(* IF YES - WRITTEN APPROVAL OF THE INSTITUTIONAL HUMAN INVESTIGATIONS COMMITTEE SHOULD BE ATTACHED.)

Medical Advisory Board
Dr. Howard L. Ait
Dr. Eric Brown
Dr. Israel Uzdsohn
Dr. Paul Heller
Dr. Leon Jacobson
Dr. Aaron M. Josephson
Dr. Stephen O. Schwartz
Dr. Irving Shulman
Dr. Paul Szanto
Dr. Stanley Yachnin

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Judge Samuel J. Ryan
Chancellor
Felix Flynn
Hugh M. Helmer
Louis Lerner
Howard Miller
Mrs. Mary G. Coppenham
Wally Phillips


SIGNATURE OF APPLICANT


SIGNATURE OF INSTITUTIONAL OFFICER

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- 2 -

6. BUDGET: (NOTE THAT GRANT IS FOR ONE YEAR.)

A. PERSONNEL: B.S. technician \$13,000* (full time)
Laboratory aide \$4,000* (one-half time)

*Salaries includes 17% fringe benefits.

B. EQUIPMENT:
None

C. SUPPLIES: \$2,200

D. OTHER EXPENSES ITEMIZED: Travel \$300

E. INSTITUTIONAL OVERHEAD:
(NOT TO EXCEED 8%) \$1,560

F. TOTAL AMOUNT REQUESTED: \$21,060

7. JUSTIFICATION FOR REQUESTED ITEMS (A, B, C, D). USE ADDITIONAL SHEETS IF NECESSARY.

A. Personnel

1. One B.S. technician is required to carry out the analytical and enzymatic assays. This person will be responsible for coordinating sample collections with the clinical chemistry and hematology laboratories.

2. One-half time laboratory aide is required for glassware washing and related service requirements associated with this project.

C. Supplies

1. Radioactive compounds (fatty acids, fatty alcohols, ethanalamine, and choline) \$1,000

2. Glassware \$300

3. Cofactors for enzyme assays \$600

4. Miscellaneous chemicals \$300

D. Other Expenses: Travel to present findings at either the FASEB meeting or the American Association for Cancer Research meeting \$300

Medical Advisory Board

Dr. Howard L. Ait
Dr. Eric Brown
Dr. Israel Davidsohn
Dr. Paul Heller
Dr. Leon Jacobson
Dr. Aaron M. Josephson
Dr. Stephen O. Schwartz
Dr. Irving Shulman
Dr. Paul Szanto
Dr. Stanley Yachnin

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Faney Flynn
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Louis Lerner
Howard Miller
Mrs. Mary G. Oppenheim
Wally Phillips

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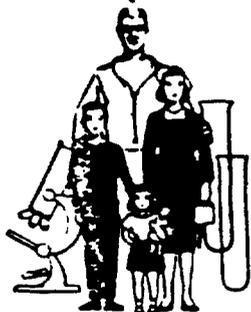
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- 3 -

8. CONCISE DESCRIPTION OF INSTITUTIONAL FACILITIES AVAILABLE FOR THIS INVESTIGATION:

Our laboratories are well equipped for biochemical investigations. Some of the most significant equipment items include an infrared spectrophotometer, liquid scintillation spectrometers, preparative ultracentrifuges with a B-XXIX zonal rotor and other conventional rotors, gas-liquid chromatographs, a thin-layer chromatography zonal scraper, automatic freeze-dryers, a liquid nitrogen refrigerator, a tissue culture room with incubators, laminar-flow hoods, Beckman DU spectrometers, photodensitometers, and a complete line of thin-layer chromatographic equipment. In addition to the equipment in our laboratories, we also make use of a nuclear magnetic spectrometer, zonal centrifuges, a mass spectrometer, an analytical ultracentrifuge, and an electron microscope in other sections of our Division and at the Oak Ridge National Laboratory. The mass spectrometer, which is connected to a gas-liquid chromatograph, is available to our group through a collaborative arrangement with Dr. W. T. Rainey, Jr., who is located at the Oak Ridge National Laboratory Analytical Division in Oak Ridge. Electron microscopic and pathologic services are also readily available in our Medical Division and at the Oak Ridge National Laboratory. Collaboration in these areas has been amply demonstrated in our published work.

9. OTHER RESEARCH SUPPORT: (LIST SHOULD ALSO INCLUDE PENDING APPLICATIONS WITH EXPLANATION OF THEIR STATUS.)

The major source of support for the Medical Division of Oak Ridge Associated Universities, including its program in biochemistry, is through a cost reimbursement contract with the United States Atomic Energy Commission (USAEC). None of the direct costs requested in this application can be authorized by the USAEC under its programmatic objectives. The laboratories and equipment with which the proposed research would be carried out are the property of the United States Government and can only be used for other work to the extent approved by USAEC. A grant from the National Institutes of Health for continued support on *Biosynthetic Mechanisms for Ether Bonds in Lipids*, May 1, 1973 through April 30, 1974 (\$24,761*) and a grant from the American Cancer Society on *The Occurrence, Metabolism, and Function of Ether-Linked Glycerolipids in Neoplasms*, December 1, 1973 through November 30, 1974 (\$47,950*) are currently funded. These funds are not available for the clinical studies outlined in this proposal.

Medical Advisory Board

Dr. Howard L. Ait
Dr. Eric Brown
Dr. Israel Davidson
Dr. Paul Heller
Dr. Leon Jacobson
Dr. Aaron M. Josephson
Dr. Stephen O. Schwartz
Dr. Irving Shulman
Dr. Paul Szanto
Dr. Stanley Yachnin

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Hon. "Arshal" Korshak
Judge Francis S. Lorent
Judge James Parsons
Judge Daniel J. Ryan
Corney Connors
Fahy Flynn
Hugh M. Helner
Louis Lerner
Howard Miller
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Wally Phillips

* Includes indirect costs.

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COUNCIL (LEUKEMIA RESEARCH FOUNDATION)

- 4 -

10. PREVIOUS GRANTS SUPPORTING THIS PROJECT: (LIST ALL GRANTS INCLUDING LEUKEMIA RESEARCH FOUNDATION, INC.)

None

Medical Advisory Board

Dr. Howard L. Ait
Dr. Eric Brown
Dr. Israel Davidsohn
Dr. Paul Heller
Dr. Leon Jacobson
Dr. Aaron M. Josephson
Dr. Stephen O. Schwartz
Dr. Irving Shulman
Dr. Paul Szanto
Dr. Stanley Yachnin

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Hon. Marshall Korshak
Judge Francis S. Lorenz
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COUNCIL (LEUKEMIA RESEARCH FOUNDATION)

- 5 -

11. CURRICULUM VITAE OF PRINCIPAL INVESTIGATOR AND ALL CO-INVESTIGATORS:
(NAME, BIRTHDATE, BIRTH PLACE, CITIZENSHIP, PROFESSIONAL EDUCATION
AND EXPERIENCE, LIST OF PUBLICATIONS FOR LAST 5 YEARS.) ATTACH
ADDITIONAL SHEETS FOR CO-INVESTIGATORS.

(See Appendix I)

Medical Advisory Board

Dr. Howard L. Ait
Dr. Eric Brown
Dr. Israel Davidsohn
Dr. Paul Heller
Dr. Leon Jacobson
Dr. Aaron M. Josephson
Dr. Stephen O. Schwartz
Dr. Irving Shulman
Dr. Paul Szento
Dr. Stanley Yachnin

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Hon. Marshall Korshak
Judge Francis S. Lorenz
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COUNCIL (LEUKEMIA RESEARCH FOUNDATION)



- 6 -

12. DESCRIPTION OF RESEARCH PROJECT: (USE AS MANY ADDITIONAL SHEETS AS ARE NECESSARY TO FURNISH FULL INFORMATION AS FOLLOWS: SUMMARY, OBJECTIVE, SIGNIFICANCE FOR LEUKEMIA RESEARCH, REFERENCES, DESCRIPTION OF CONTRIBUTION OF OTHERS IN THIS FIELD OF RESEARCH.)

INCLUDE A CONCISE ONE PARAGRAPH DESCRIPTION, IN LAY TERMS, OF YOUR RESEARCH PROJECT AND ITS POTENTIAL FOR FURTHERING UNDERSTANDING AND KNOWLEDGE OF LEUKEMIA.

A. Background

During the mid-1960's, our group detected and identified significant quantities of the ether-linked glycerolipids in a variety of neoplastic cells from animals and humans. These rather unusual lipid structures are not prevalent in normal tissues; the enclosed figure (Appendix II) indicates the presence of alkyldiacylglycerols in tumors and their absence in normal cells. Our laboratory has elucidated the enzymic pathways responsible for the synthesis of ether lipids and we have demonstrated that these reactions represent a prominent biosynthetic route in neoplastic cells of both animal and human origins. Although the significance of the high levels of ether-linked lipids in neoplasms is unknown, these lipids appear to be closely connected with malignancy, i.e., they are highest in the most malignant cells. The extremely stable ether-linked lipids might be related to the properties of the surface membranes of metastatic cells that make them resistant to catabolic enzymes, e.g., phospholipases.

B. Summary of Project and Objectives

We would like to apply our basic expertise to evaluate the usefulness of the ether-linked lipids and their enzymes as potential markers for clinical specimens at various stages of malignancy. In the past we have only had support for limited experiments and, therefore, we have only been able to contribute a small amount of staff time to these potentially important clinical aspects of the problem. Some recent preliminary work with leukemic cells (see attached Appendix III) from patients with chronic lymphocytic leukemia and chronic granulocytic leukemia has indicated that such cells show the same characteristic increase in ether-linked lipids and their biosynthetic enzymes that have been apparent in all of our experiments with transplantable, chemically induced, and virally induced animal tumors. We believe that support for detailed studies in this field with clinical material could yield important results in the area of early cancer detection.

In the proposed investigation we would assay O-alkyl and O-alk-1-enyl synthesizing enzymes in whole blood and in cellular material (total and purified cell types) from normal individuals and patients afflicted with chronic and acute forms of leukemia. In connection with these enzyme assays, we also plan to carry out a detailed analysis of the lipid classes and their fatty acid composition to see if any distinguishing characteristics can be detected at various stages of development and treatment of different types of leukemia.

Medical Advisory Board

Dr. Howard L. Art
Dr. Eric Brown
Dr. Israel Davidson
Dr. Paul Heller
Dr. Leon Jacobson
Dr. Aaron M. Josephson
Dr. Stephen O. Schwartz
Dr. Irving Shuiman
Dr. Paul Szanto
Dr. Stanley Yechnin

Honorary Board

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12.

A sizable segment of the clinical group's effort at the Medical Division deals with patients who have leukemia or other hematologic malignancies. Therefore, we anticipate that a large series of patients with different types of leukemia can be evaluated in this project. Additional clinical samples are also available from hospitals in Knoxville through arrangements with the East Tennessee Cancer Research Center. Since the clinical chemistry group is under the direction of our Biological Chemistry Department, we will also have excellent access to samples from patients and healthy individuals who do not have hematopoietic disorders. The aim of this proposal is to exploit all facets of ether lipid metabolism in normal and malignant blood cells from humans to see if any of the enzymes, precursors, or products involved in these pathways presage the development of leukemia. The methodology that will be used is described in following sections.

C. Summary of Methodology

1. Extraction, chromatography, and analyses of lipids

Total lipids will be extracted and purified from lyophilized tissues with chloroform:methanol (2:1, v/v) by the procedure of Bligh and Dyer (1). The alkyl- and alk-1-enyl-glycerols, liberated from neutral or phosphoglyceride fractions and individual lipid classes by LiAlH_4 reduction and separated by thin-layer chromatography (2), will be quantitated by photodensitometry. We will use the procedure described by Van Golde and Van Deenen (3) to determine molecular species of phospholipids in the various cell types. The mass of all major lipid classes will be quantitated by photodensitometry (4) or by determining the phosphorus content (5). Alkyl- and alk-1-enyl-glycerols formed by LiAlH_4 reduction of individual lipid classes will be purified by preparative chromatography on Silica Gel G in a solvent system of diethyl ether saturated with water. In metabolic experiments with radioactive precursors, the distribution of radioactivity in lipid classes will be quantitated by scanning entire chromatographic lanes in 2-mm increments (6).

Isopropylidene derivatives of the alkylglycerols (7), fatty aldehydes liberated from alk-1-enylglycerols (8), acetates of the fatty alcohols (9), and methyl esters of the fatty acids (9) will be prepared and quantitated by gas-liquid chromatography. Gas-liquid chromatography will resolve these derivatives according to chain length and degree of unsaturation on 10% EGSS-X coated on Gas-Chrom P under conditions identical to those described (9). A Victoreen 4000 series gas-liquid chromatograph equipped with a dual hydrogen flame will be used for these analyses; radioactivity will be collected in glass micropipettes (filled with glass wool saturated with chloroform) attached to a splitter (9:1) at the end of the column. The radioactivity collected in the micropipettes will be quantitatively transferred to a vial with chloroform and the solvent removed under vacuum. The radioactivity will be measured in liquid scintillation spectrometers after a scintillator fluid is added (10). The principal chemical procedures used to study alkyl and alk-1-enyl lipids isolated from metabolic systems are summarized in the two schemes shown below.

12.

References

1. Bligh, E. G. and Dyer, W. J. *Can. J. Biochem. Physiol.* 37: 911 (1959).
2. Wood, R. and Snyder, F. *Lipids* 3: 129 (1968).
3. Van Golde, L. M. G. and Van Deenen, L. L. M. *Biochim. Biophys. Acta* 125: 496 (1966).
4. Privett, O. S. and Blank, M. L. *J. Amer. Oil Chem. Soc.* 39: 520 (1962).
5. Rouser, G., Siakotos, A. N., and Fleischer, S. *Lipids* 1: 85 (1966).
6. Snyder, F. and Kimble, H. *Anal. Biochem.* 11: 510 (1965).
7. Hanahan, D. J., Ekholm, J., and Jackson, C. M. *Biochemistry* 2: 630 (1963).
8. Wood, R. and Harlow, R. *J. Lipid Res.* 4: 463 (1969).
9. Snyder, F. and Blank, M. L. *Arch. Biochem. Biophys.* 130: 101 (1969).
10. Snyder, F., Blank, M. L., Malone, B., and Wykle, R. L. *J. Biol. Chem.* 245: 1800 (1970).

2. Biochemical procedures

Organelles will be prepared by well known and established conventional and zonal centrifugation procedures that have been used in our laboratories for many years. For enzymic assays, we will use the basic incubation system described for the biosynthesis (1) or biocleavage (2,3) of alkylglycerols. The biosynthesis of plasmalogens in similar systems will be investigated (4). The cleavage of plasmalogens will also be assayed (5,6).

References

1. Snyder, F., Malone, B., and Blank, M. L. *J. Biol. Chem.* 245: 1790 (1970).
2. Tietz, A., Lindberg, M., and Kennedy, E. P. *J. Biol. Chem.* 239: 4081 (1964).
3. Soodsma, J. F., Piantadosi, C., and Snyder, F. *J. Biol. Chem.* 247: 3923 (1972).
4. Wykle, R. L., Blank, M. L., Malone, B., and Snyder, F. *J. Biol. Chem.* 247: 5442 (1972).
5. Warner, H. R. and Lands, W. E. M. *J. Biol. Chem.* 236: 2404 (1961).
6. Ansell, G. B. and Spanner, S. *Biochem. J.* 94: 252 (1965).

D. Relationship of the Contributions of Others to This Field

Since the discovery of enzymic systems that synthesize alkyl glycerolipids (1,2), other similar systems have also been found in a variety of preparations of biological origin (3,4). Biosynthesis of the O-alkyl linkage occurs when DHAP and fatty alcohols are incubated with CoA, ATP, Mg⁺⁺, and the enzyme source. However, Hajra (5) and later our laboratory (6), demonstrated that the acyl moiety of acyl-DHAP can be displaced by free fatty alcohols in the presence of Mg⁺⁺. Murooka and colleagues (7) have also obtained data for the alkylation of homoserine that supports the principle of this mechanism. We have determined that the entire chain of the alcohol (9), including the oxygen (10), replaces the acyl group.

The potential precursor role of O-alkyl lipids in plasmalogen biosynthesis had been implicated from a number of in vivo studies carried out with ³H/¹⁴C-labeled alkylglycerols (11,12) and [1-¹⁴C]-1-alkyl-2-acyl-*sn*-glycero-3-phosphoryl-ethanolamine (13). Recent enzyme studies have now shown that alk-1-enyl (plasmalogen) linkages in glycerolipids originate from the alkyl grouping via a mixed-function oxidase in tumors (14) and intestinal mucosa (15). The substrate is an intact alkylethanolamine-containing phosphatide (1-alkyl-2-acyl-*sn*-glycero-3-phosphorylethanolamine) and the requirements for the reaction are molecular oxygen and NADPH; a soluble cytoplasmic factor, ATP, and Mg⁺⁺ also stimulate this conversion. The cytochrome b₅ electron transport system instead of the P-450 system has been implicated for the alkyl desaturase (14) and in this respect is similar to that found for fatty acid desaturase (16).

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E. Relevance of Project to Furthering Our Understanding and Knowledge of Leukemia

In addition to the usual ester bonds in lipids produced by most healthy cells, cancer cells produce lipid components that also contain significantly higher quantities of ether bonds than that found in normal cells. During the past 5 years our group has been able to determine the enzyme sequence in cancer cells that accounts for the formation of the complex ether-linked lipids from simple precursors, fatty alcohols and dehydroxyacetone-P. We have also shown that the alcohols themselves are derived from certain long-chain fatty acids in the tumors. The other precursor, dehydroxyacetone-P is produced from glucose, when it is used as an energy source. Results obtained with animal tumors and tissue culture systems have indicated that the metabolic changes that cause the ether lipids to build up in tumor cells occur at an early stage of the neoplastic process and that the enzymes involved might be unique sensitive biochemical markers for the early detection of leukemia and could also be useful markers for monitoring the disease during therapy. The clinical facilities at the Medical Division of Oak Ridge Associated Universities and other hospitals participating in the East Tennessee Cancer Research Center will be the source of blood samples from patients with leukemia and other hematologic malignancies, as well as those samples obtained from healthy individuals. Both the biochemical and hematologic results will be carefully correlated. The ultimate goal is to determine whether any of the newly discovered enzymes involved in the metabolism of ether-linked lipids can be used to characterize early malignant changes that occur in human blood cells.

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Although the major emphasis of this project is directed toward an enzymatic diagnostic approach for leukemia, we expect to obtain new fundamental knowledge about the leukemic process. A sound understanding of ether lipids and membranes in leukemic leukocytes could lead to the development of analogs of metabolic intermediates that have therapeutic potential.

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APPENDIX I (-1-)

CURRICULUM VITAE

FRED SNYDER

[REDACTED] married, 3 children.

Education

[REDACTED]

Positions

- 1958-present: Chief Scientist, Medical Division, Oak Ridge Associated Universities, Oak Ridge, Tennessee.
- 1964-present (joint appointment): Professor of Biochemistry, University of Tennessee Medical School, Memphis, Tennessee.
- 1966-present (joint appointment): Professor of Medicinal Chemistry, University of North Carolina, Chapel Hill, North Carolina.
- 1971-1974: Associate Editor, Cancer Research.
- 1972- : Member of Editorial Board of Archives of Biochemistry and Biophysics.
- 1966-present: Member of Editorial Board of the Journal of Lipid Research.
- 1973-1977: Member of Editorial Board of Biochimica et Biophysica Acta.
- 1973- : Member of Editorial Board of Biochimica et Biophysica Acta — Reviews on Cancer.
- 1968-1972: Member of the American Cancer Society Advisory Committee on Biochemistry and Chemical Carcinogenesis.
- 1972-1973: Chairman, Southeastern Section of the Society for Experimental Biology and Medicine.
- 1969-1970: Secretary-Treasurer, Southeastern Section of the Society for Experimental Biology and Medicine.
- 1965-1968: Sectional Councilor, Southeastern Section of the Society for Experimental Biology and Medicine.
- 1964, Fall Semester: Visiting Scientist, University of North Carolina, Chapel Hill, North Carolina.
- 1955-1958: Predoctoral Fellowship, National Heart Institute.

Societies

- American Society of Biological Chemists
- American Association for Cancer Research, Inc.
- American Chemical Society
- New York Academy of Science
- Radiation Research Society
- Sigma Xi
- Society for Experimental Biology and Medicine

Publications

Total number of full-length publications in biochemical journals or books is over 150. Representative publications since 1969 are on the attached list.

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PARTIAL
LIST OF PUBLICATIONS

Fred Snyder

1969-1974

1974

Blank, M. L. and Snyder, F.

A Tissue Culture System for Studies of the Regulation of Ether-Linked and Ester-Linked Aliphatic Moieties in Glycerolipids.

Arch. Biochem. Biophys., 160, 100-105, 1974.

Blank, M. L. and Snyder, F.

Qualitative and Quantitative Aspects of Thin-Layer Chromatography of Neutral Lipids.

J. Amer. Oil Chem. Soc., in press

Snyder, F.

Analysis of Alkyl and Alk-1-enyl Ether Lipids and their Derivatives by Chromatographic Techniques.

Chapter __, Lipid Chromatographic Analysis, (G. V. Marinetti, ed.)

Marcel Dekker, New York, in press.

Snyder, F. and Snyder, C.

Glycerolipids and Cancer

Chapter __, Progress in Biochemical Pharmacology, Vol. 9 (Lipids in Tumors), (K. K. Carroll, Ed.) S. Karger, New York, in press.

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Arch. Biochem. Biophys., in press.

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Biochem. Biophys. Res. Commun., 55, 574-579, 1973.

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Arch. Biochem. Biophys., 154, 648-658, 1973.

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Biochim. Biophys. Acta, 291, 71-82, 1973.

Lee, T-c., Stephens, N., Moehl, A., and Snyder, F.

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Biochem. Biophys. Res. Commun., 51, 119-124, 1973.

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Biochem. Biophys. Res. Commun., 53, 350-356, 1973.

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 Measurement and the Occurrence and Nature of the Alkyl and
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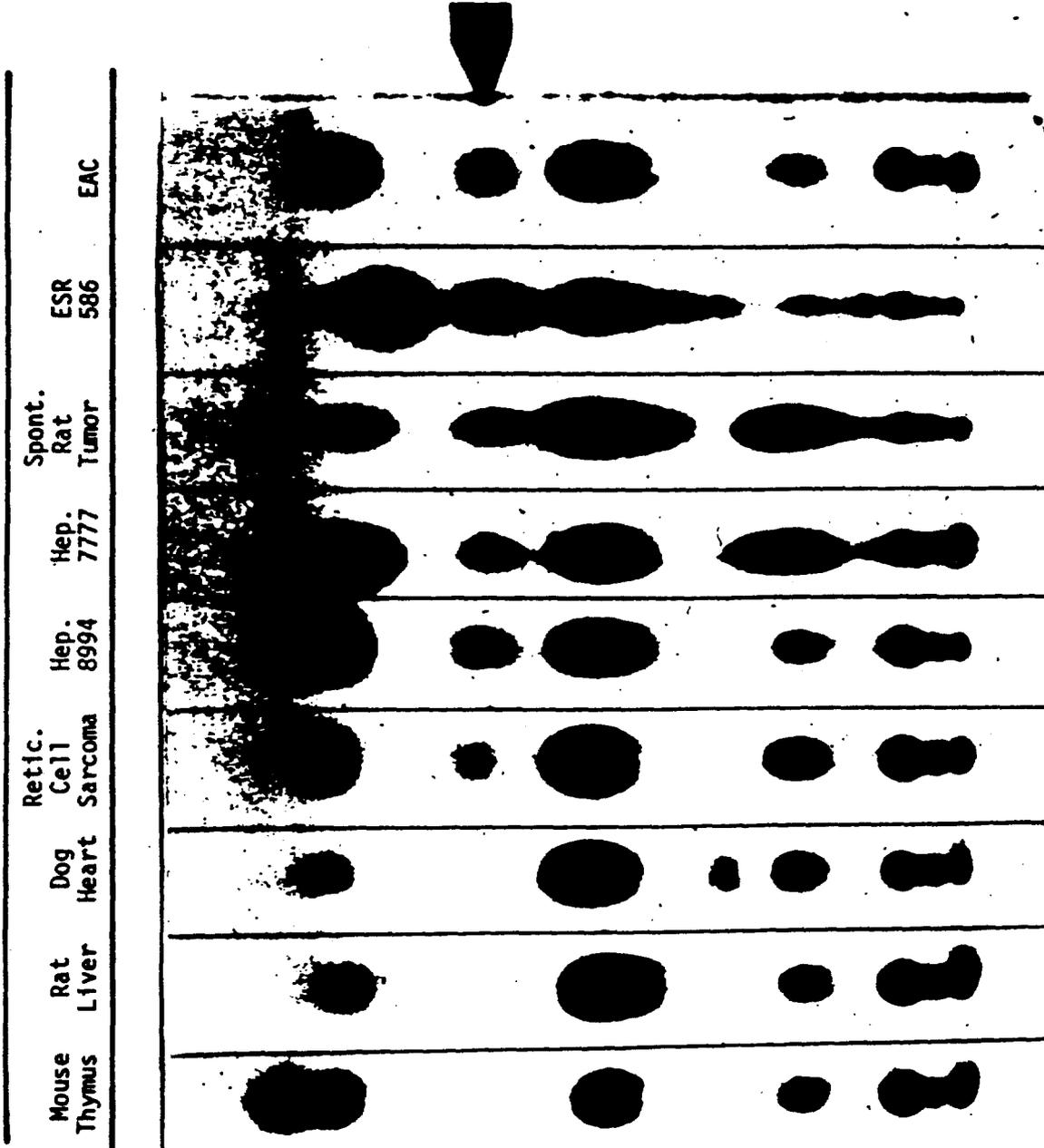
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1036374

NORMAL AND TUMOR TISSUE LIPIDS



1036375

FIGURE LEGEND

Representative thin-layer chromatogram of total lipids from normal tissues and tumors. The abbreviated notations above each lane designate reticulum-cell sarcoma (human), Morris hepatoma 8994 (rat), Morris hepatoma 7777 (rat), spontaneous rat tumor, preputial gland tumor ESR-586 (mouse), and Ehrlich ascites cells (mouse). The arrow indicates the location of the alkyldiacylglycerols; the spots directly below the alkyldiacylglycerols are triacylglycerols. A solvent mixture of hexane:diethyl ether:acetic acid (90:10:1, v/v) was used for development, and Silica Gel G was the adsorbent.

[From: *Ether Lipids: Chemistry and Biology* (F. Snyder, Ed.), Academic Press, pp-273-295, 1972.]

Biochemical Studies of Human Leukemic Cells

We have recently initiated a project on lipid metabolism in blood cells from patients with leukemia and erythrocythemic disorders (M. L. Blank, M. Clevenger, F. A. Goswitz, B. Malone, and F. Snyder). The primary aim of this work is to determine whether human blood cells considered malignant have characteristic abnormalities of lipid metabolism that are seen in nonhematologic malignancies. The blood samples under investigation include intact cells, homogenates, and subcellular fractions from lymphocytes, granulocytes, erythrocytes, platelets, and whole blood.

Preliminary results from eleven different patients have demonstrated that alkyl glycerolipids are synthesized from long-chain fatty alcohols by intact leukemic lymphocytes and granulocytes and their cell-free homogenates. Typical data are depicted in Table 44. Maximal synthesis occurred after about 1 hr of incubation. The ether-linked aliphatic moieties of the glycerolipids synthesized by leukocytes from patients with chronic granulocytic and lymphocytic

Table 44
BIOSYNTHESIS OF ALKYL GLYCEROLIPIDS
BY CELL-FREE HOMOGENATES
OF LEUKEMIC CELLS*

Patient number	Picamoles per mg protein	Picamoles per million cells
1	477	12.7
2	649	23.7
3	842	37.3
4	495	34.0

*Other cell fractions did not synthesize alkyl glycerolipids. Incubations contained dihydroxyacetone-P, 1-¹⁴C-hexadecanol, CoA, ATP, Mg⁺⁺, and homogenate in phosphate buffer at pH 7.1. All values have been corrected for appropriate cofactor controls.

leukemias are similar to those found in nonhematologic neoplasms (mainly 16:0, 18:0, and 18:1) except that the leukemic cells also contain a significant quantity of longer carbon chains (Tables 45 and 46). Our data indicate that the leukemic cell is an excellent system for investigating the ether-lipid pathways and their regulation in human subjects with neoplasms. Continued work on this project is contemplated.

From the 1971 Research Report, Medical Division, Oak Ridge Associated Universities, pp. 127-128, United States Atomic Energy Commission ORAU-116, 1972.

Table 45

CHRONIC LYMPHOCYTIC LEUKEMIA: COMPOSITION OF O-ALKYL AND O-ALK-1-ENYL MOIETIES OF GLYCEROLIPIDS* FROM LYMPHOCYTES AND ERYTHROCYTES

Chain length	Alkyl		
	Lymphocytes	Erythrocytes	Erythrocytes
16:0	28.8	28.0	25.6
17:0 + 16:1 + 17:1	1.9	2.0	2.7
18:0	15.4	29.5	44.8
18:1	32.0	25.6	21.4
18:2	tr	tr	1.0
20:0	1.6	1.2	1.1
20:1	2.9	1.3	1.0
20:2	1.2	tr	tr
22:0	tr	tr	tr
22:1	16.2	12.4	tr
A	tr	tr	2.4

*Derived from total lipids extract

Table 46

CHRONIC GRANULOCYTIC LEUKEMIA: COMPOSITION OF O-ALKYL AND O-ALK-1-ENYL MOIETIES OF GLYCEROLIPIDS FROM GRANULOCYTES AND ERYTHROCYTES

Chain length	Alkyl		
	Granulocytes	Erythrocytes	Erythrocytes
16:0	22.8	19.3	23.2
17:0 + 16:1 + 17:1	2.8	Present	1.7
18:0	20.1	18.2	44.5
18:1	29.2	38.5	16.7
18:2	tr	tr	tr
20:0	3.7	tr	1.7
20:1	2.7	tr	2.6
20:2	2.4	tr	tr
22:0	1.7	tr	tr
22:1	12.2	24.0	tr
A	2.4	tr	9.6

5.5*

1036377

Ident. No. _____

APPLICATION FOR THE USE OF HUMANS AS EXPERIMENTAL SUBJECTS

To: **COMMITTEE ON HUMAN STUDIES**
Oak Ridge Associated Universities and
Oak Ridge National Laboratory

Date 11 February 1974

Principal Investigator: Fred L. Snyder

Co-Investigators: _____

Title of Project: LIPID MARKERS IN HUMAN LEUKEMIC CELLS

Use Following Format (Submit Original and 8 Copies)

I. Objectives of Experiment:

(Include statement why experiment must be done in humans, and expected benefits from the knowledge.)

II. Methods of Procedure:

Brief description of methods, all medications including name and dose range, number and types of subjects anticipated, time for single session, total number of sessions, total duration of study, methods used to screen subjects, etc.

III. Possible Hazards and their Evaluation:

IV. Radioisotopes and New Drugs

If the study involves radioisotopes, indicate action of the Isotopes Committee. If new drugs are involved, indicate that appropriate application to FDA has been made.

See page 2

1036378

I. Objectives of Experiment

In the proposed investigation we plan to assay O-alkyl and O-alk-1-enyl synthesizing enzymes in whole blood and in purified cells from normal individuals and patients afflicted with chronic and acute forms of leukemia. In connection with these enzyme assays, we also plan to carry out a detailed analysis of the lipid classes and their fatty acid composition to see if any distinguishing characteristics can be detected at various stages of development in different types of leukemia.

II. Methods of Procedure

Only blood samples will be analyzed. Typical volumes will range from 5 to 50 ml depending on the type of analysis to be carried out. The total number of samples will depend on the number of patients available.

III. Possible Hazards and their Evaluation

No hazards will be involved, since the analyses will require blood sampling procedures that are identical to those used for other clinical chemistry assays.

IV. Radioisotopes and New Drugs

None.

Title of Project: LIPID MARKERS IN HUMAN LEUKEMIC CELLS

Ident. No. _____

V. Responsibility of Principal Investigator:

Include statement of your procedures for protecting rights of the patients and gaining informed consent.

The principal investigator will follow the procedures of the Committee on Human Studies in obtaining "informed consent" from the subjects under study. The investigator recognizes that he retains the primary responsibility for safe-guarding the interests of the participants under study. Any significant changes in methods of procedure or of the development of unexpected risks will be brought to the attention of the Committee on Human Studies.

Starting Date 1 July 1974

Signatures: *Earl Snyder* Principal Investigator

_____ Co-Investigator

_____ "

_____ "

_____ "

_____ "

DIVISION REVIEW:

The application described above has been reviewed and approved.

Official signing for the institution:

Signature *Garret Andrews*

Title Chairman, Medical Division

Institution Oak Ridge Associated Universities

Date February 11, 1974

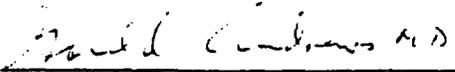
1036380

(Revised January 1972)

February 11, 1974

Leukemia Research Foundation, Inc.
Chicago, Illinois

As chairman of our committee on Human Studies I am able to state that this committee will approve of Dr. Snyder's application. Since it involves only studies on blood samples of modest size, it is not necessary for the committee to meet in advance of sending the application. However, we will meet and approve the work before the work actually starts.



Gould A. Andrews, M.D.

1036381