Dear Dr. Pollard:

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

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

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

ROSE: dh

1036349
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

SLK:s
cc: Mr. W. C. Pollard

1036350
REMEMBER, THERE'S NO CURE FOR LEUKEMIA... YET!
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:

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:wf1  
Enclosures 0
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

C. W. HILL

ORR: JDB

Enclosure: Proposal

CC: C. W. HILL
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,

William G. Pollard
Executive Director

Enclosures

bc: Mr. Lenhard, AEC/ORO
   Executive Office
   Mr. Rose     Dr. Andrews     Dr. Snyder     Mr. Crockett
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.)

SIGNATURE OF APPLICANT

SIGNATURE OF INSTITUTIONAL OFFICER

1036354
REMEMBER, THERE'S NO CURE FOR LEUKEMIA... YET!
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, ethanolamine, 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

REMEMBER, THERE'S NO CURE FOR LEUKEMIA...YET!
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.

3. 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.

* Includes indirect costs.

1036356

REMEMBER, THERE'S NO CURE FOR LEUKEMIA... YET!
10. PREVIOUS GRANTS SUPPORTING THIS PROJECT: (LIST ALL GRANTS INCLUDING LEUKEMIA RESEARCH FOUNDATION, INC.)

None
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)
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 alkyl diacylglycerols 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.
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 LiAlH₄ 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 LiAlH₄ 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.
Analysis of alkyl glycerolipids

Analysis of alk-1-enyl glycerolipids (plasmalogens)
12.

References


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

   (1972).

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-phosphorylthanolamine) 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).
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 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.
12.
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.
APPENDIX I

CURRICULUM VITAE

FRED SNYDER

Education

Married, 3 children.

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.


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.

1036365
APPENDIX I

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.

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

Snyder, F., Malone, B., and Piantadosi, C.
Enzymic Studies of Alkyl and Glycerol Lipids containing Alkyl Bonds in Liver and Tumor Tissues.
Arch. Biochem. Biophys., in press.

1973

Snyder, C., Malone, B., Nettesheim, P., and Snyder, F.
Urethan-Induced Pulmonary Adenoma as a Tool for the Study of Surfactant Biosynthesis.

Chae, K., Piantadosi, C., and Snyder, F.
Reductase, Phosphatase, and Kinase Activities in the Metabolism of Alkylidihydroxyacetone Phosphate and Alkylidihydroxyacetone.

Snyder, F., Malone, B., and Piantadosi, C.
Tetrahydropteridine-Dependent Cleavage Enzyme for 0-Alkyl Lipids: Substrate Specificity.

Wykle, R. L., Blank, M. L., and Snyder, F.
The Enzymic Incorporation of Arachidonic Acid into Ether-Containing Choline and Ethanolamine Phosphoglycerides by Deacylation-Acylolation Reactions.

Lee, T.-C., Wykle, R. L., Blank, M. L., and Snyder, F.
Dietary Control of Stearyl CoA and Alkylacylglycerolphosphorylethanolamine Desaturation in Tumor
1973

Piantadosi, C., Chae, K., Ishaq, K. S., and Snyder, F.
Chemical Synthesis of $1^{-14}C$-Octadecylidihydroxyacetone Phosphate and
$1^{-14}C$-Octadecylidihydroxyacetone.

Kasama, K., Rainey, W. T., Jr., and Snyder, F.
Chemical Identification and Enzymatic Synthesis of a Newly Discovered
Lipid Class — Hydroxyalkylglycerols.

Lee, T-c., and Snyder, F.
Phospholipid Metabolism in Rat Liver Endoplasmic Reticulum:
Structural Analyses, Turnover Studies, and Enzymic Activities.

Turnover of Rat Liver Plasma Membrane Phospholipids: Comparison with
Microsomal Membranes.

Chae, K., Piantadosi, C., and Snyder, F.
An Alternate Enzymic Route for the Synthesis of the Alkyl Analog of
Phosphatidic Acid Involving Alkylglycerol.

Snyder, F.
Thin-Layer Chromatographic Behavior of Glycerolipid Analogs Containing
Ester, Ester, Hydroxyl, and Ketone Groupings.

(Presented as an invited lecture at the 2nd International CMAG Symposium on
Advanced Thin-Layer Chromatography and Electrophoresis, New York, November 1972.)

Snyder, F.
Radioisotopes — Introduction. Detection and Chromatographic Resolution
of Labeled Lipid Intermediates Formed in Enzyme Systems.

(Presented as an invited lecture at the 5th International Symposium on
Quantitative Flat-Bed Chromatography, Carlsbad, Czechoslovakia, September 1972.)

Blank, M. L., Wykle, R. L., and Snyder, F.
The Retention of Arachidonic Acid in Ethanolamine Plasmalogens of Rat
Testes during Essential Fatty Acid Deficiency.

Grigor, M. R., Blank, M. L., and Snyder, F.
Cholesterol Metabolism in Rats Bearing Morris Hepatoma 7777.

Snyder, F., Clark, M., and Piantadosi, C.
Biosynthesis of Alkyl Lipids: Displacement of the Ayl Moiety of Acylhydroxy-
acetone Phosphate with Fatty Alcohol Analogs.
Snyder, F.
Enzymic Systems that Synthesize and Degrade Glycerolipids Possessing Ether Bonds.

Wykle, R. L., Piantadosi, C., and Snyder, F.
The Role of Acylidihydroxyacetone Phosphate, NADH, and NADPH in the Biosynthesis of 0-alkyl Glycerolipids by Microsomal Enzymes of Ehrlich Ascites Tumor.

Blank, M. L., Kasama, K., and Snyder, F.
Isolation and Identification of an Alkylidiaclylglycerol Containing Isovaleric Acid.

Piantadosi, C., Ishaq, K. S., Blank, M. L., and Snyder, F.
Synthesis of 1-0-Alkyl-3-0-Phosphorylcholine-2-Propanone and Related Derivatives.

Snyder, F.
Ether-Linked Lipids and Fatty Alcohol Precursors in Neoplasms.

Snyder, F.
Enzymic Pathways of Ether-Linked Lipids and Their Precursors.

Grigor, M. R., Pratt, R. D., and Snyder, F.
Acetylation of Sterol by Microsomal Enzymes from Mouse Preputial Glan Tumors.

Piantadosi, C., Chae, K., Ishaq, K. S., and Snyder, F.
Synthesis of Acylidihydroxyacetone Phosphates and Related Derivatives.

Soodsma, J. F., Piantadosi, C., and Snyder, F.
Partial Characterization of the Alkylglycerol Cleavage Enzyme System of Rat Liver.

Blank, M. L., Wykle, R. L., and Snyder, F.
The Biosynthesis of Ethanolamine Plasmalogens by a Postmitochondrial Fraction from Rat Brain.

Wykle, R. L., Blank, M. L., Malone, B., and Snyder, F.
Evidence for a Mixed-Function Oxidase in the Biosynthesis of Ethanolamine Plasmalogens from 1-Alkyl-2-Acyl-sn-Glycerol-3-Phosphorylcholine.

Grigor, M. R., Meel, A., and Snyder, F.
Occurrence of Ethanolamine- and Choline-Containing Plasmalogens in Adipose Tissue.
Lipids, 7, 766-768, 1972.
1971

Grigor, M. R., Blank, M. L., and Snyder, F.
Structural Relationships between Glycerides of Pig Serum and Adipose Tissue.
Lipids, 6, 965-968, 1971.

Snyder, F., and Moehl, A.

Piantadosi, C., Ishaq, K. S., Wykle, R. L., and Snyder, F.
Synthesis and Characterization of 1-O-Alkylidihydroxyacetone Phosphates and Derivatives.
Biochemistry, 10, 1417-1421, 1971.

Snyder, F., Blank, M. L., and Wykle, R. L.
The Enzymic Synthesis of Ethanolamine Plasmalogens.

Snyder, F., Hibbs, M., and Malone, B.
Enzymic Synthesis of O-Alkyl Glycerolipids in Brain and Liver of Rats during fetal and Postnatal Development.

Bell, O. E., Jr., Blank, M. L., and Snyder, F.
The Incorporation of $^{14}C$ from Long Chain Alcohols into the Alkyl and Alk-1-ethyl Ethers of Phospholipids of Developing Rat Brain.

On the Analysis of Long Chain Alkane Diols and Glycerol Ethers in Biochemical Studies.

Lumb, R. H., and Snyder, F.
A Rapid Isotopic Method for Assessing the Biosynthesis of Ether Linkages in Glycerolipids of Complex Systems.

Blank, M. L., Wykle, R. L., and Snyder, F.
Enzymic Synthesis of Ethanolamine Plasmalogens from an O-Alkyl Glycerolipid.

Snyder, F.

Snyder, F.
Glycerolipids in the Neoplastic Cell: Methodology, Metabolism, and Complication.
1970

Snyder, F., Piantadosi, C., and Malone, B.
The Participation of 1- and 2-Isomers of 0-Alkylglycerols as Acyl Acceptors in Cell-Free Systems.
Biochim. Biophys. Acta, 202, 244-249, 1970.

Sansone, G., Swartzendruber, D. C., and Snyder, F.
Inclusions in Mitochondria of Preputial Glands from Mice. A Combined Biochemical and Morphologic Study.

Snyder, F., Malone, B., and Blank, M. L.
Enzymic Synthesis of O-Alkyl Bonds in Glycerolipids.

Snyder, F., Blank, M. L., Malone, B., and Wykle, R. L.
Identification of O-Alkylidihydroxyacetone Phosphate, O-Alkylidihydroxyacetone, and Diacyl Glycerol Ethers after Enzymic Synthesis.

Wykle, R. L., and Snyder, F.
Biosynthesis of an O-Alkyl Analogue of Phosphatidic Acid and O-Alkylglycerols via O-Alkyl Ketone Intermediates by Microsomal Enzymes of Ehrlich Aspirate Tumor.

Snyder, F., Blank, M. L., and Malone, B.
Requirement of Cytidine Derivatives in the Biosynthesis of O-Alkyl Phospholipids.

Piantadosi, C., Ishaq, K. S., and Snyder, F.
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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 alkyl-diacyl-glycerols; the spots directly below the alkyl-diacyl-glycerols are triacyl-glycerols. 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.

Biochemical Studies of Human Leukemic Cells

We have recently initiated a project on lipid metabolism in blood cells from patients with leukemia and erythrothrombocytopenic disorders (M. L. Blank, M. Clevenger, F. A. Gospiz, 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 leukemia 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 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.


Table 45
CHRONIC LYMPHOCYTIC LEUKEMIA: COMPOSITION OF O-ALKYL AND O-ALK-1-ENYL MOIETIES OF GLYCEROLIPIDS*
FROM LYMPHOCYTES AND ERYTHROCYTES

<table>
<thead>
<tr>
<th>Chain length</th>
<th>Lymphocytes</th>
<th>Erythrocytes</th>
<th>Lymphocytes</th>
<th>Erythrocytes</th>
</tr>
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<tbody>
<tr>
<td>16:0</td>
<td>28.8</td>
<td>28.0</td>
<td>27.4</td>
<td>25.6</td>
</tr>
<tr>
<td>17:0 + 16:1 + 17:1</td>
<td>1.9</td>
<td>2.0</td>
<td>3.4</td>
<td>2.7</td>
</tr>
<tr>
<td>18:0</td>
<td>15.4</td>
<td>29.5</td>
<td>29.2</td>
<td>44.8</td>
</tr>
<tr>
<td>18:1</td>
<td>32.0</td>
<td>25.6</td>
<td>28.9</td>
<td>21.4</td>
</tr>
<tr>
<td>18:2</td>
<td>tr</td>
<td>tr</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>20:0</td>
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<td>1.2</td>
<td>1.3</td>
<td>1.1</td>
</tr>
<tr>
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<td>2.9</td>
<td>1.3</td>
<td>1.9</td>
<td>1.0</td>
</tr>
<tr>
<td>20:2</td>
<td>1.2</td>
<td>tr</td>
<td>1.7</td>
<td>tr</td>
</tr>
<tr>
<td>22:0</td>
<td>tr</td>
<td>tr</td>
<td>tr</td>
<td>tr</td>
</tr>
<tr>
<td>22:1</td>
<td>16.2</td>
<td>12.4</td>
<td>tr</td>
<td>tr</td>
</tr>
<tr>
<td>A</td>
<td>tr</td>
<td>3.3</td>
<td>2.4</td>
<td></td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>Chain length</th>
<th>Granulocytes</th>
<th>Erythrocytes</th>
<th>Granulocytes</th>
<th>Erythrocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>22.8</td>
<td>19.3</td>
<td>26.0</td>
<td>23.2</td>
</tr>
<tr>
<td>17:0 + 16:1 + 17:1</td>
<td>2.8</td>
<td>Present</td>
<td>2.0</td>
<td>1.7</td>
</tr>
<tr>
<td>18:0</td>
<td>20.1</td>
<td>18.2</td>
<td>39.9</td>
<td>44.5</td>
</tr>
<tr>
<td>18:1</td>
<td>29.2</td>
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<td>12.1</td>
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</tr>
<tr>
<td>18:2</td>
<td>tr</td>
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<td>tr</td>
<td></td>
</tr>
<tr>
<td>20:0</td>
<td>3.7</td>
<td>tr</td>
<td>5.5*</td>
<td>1.7</td>
</tr>
<tr>
<td>20:1</td>
<td>2.7</td>
<td>tr</td>
<td>2.9</td>
<td>2.6</td>
</tr>
<tr>
<td>20:2</td>
<td>2.4</td>
<td>tr</td>
<td>2.7</td>
<td>tr</td>
</tr>
<tr>
<td>22:0</td>
<td>1.7</td>
<td>tr</td>
<td>1.1</td>
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<tr>
<td>22:1</td>
<td>12.2</td>
<td>24.0</td>
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</tr>
<tr>
<td>A</td>
<td>2.4</td>
<td>tr</td>
<td>4.3</td>
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</tbody>
</table>

*From total lipids extract.
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

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
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

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: [Signature] Principal Investigator

[Signature] Co-Investigator

DIVISION REVIEW:

The application described above has been reviewed and approved.

Official signing for the institution:

Signature [Signature]

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.