Page 1 of 13

EXHIBIT 2 Part 1 of 3

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ABSTRACT OF RESEARCH PLAN

HAME AND ADDRESS OF APPLICANT ORGANIZATION (Some as few 21, page 1)

The University of Chicago, 5801 S. Ellis Avenue, Chicago, Illinois 60637

TITLE OF APPLICATION (Some on Item 1, page 1)

Frythropoletin: Purification, Properties, Ringenesis, James, Title and Department of all professional personnel angused on project, designing of and angued on project, beginning with Principal Investigator/Program Director

Eugene Goldwasser, SS # 494-14-6535, Department of Biochemistry Fung-Fang Wang, SS # 135-52-9600, Department of Biochemistry

ABSTRACT OF RESEARCH PLAN: Concisely describe the application's specific aims, methodology and languarm objectives, making reference to the scientific disciplines involved and the health-relatedness of the project. The obstract should be self-contained so that it can serve as a succinct and accurate description of the application when separated from it. DO NOT EXCEED THE SPACE PROVIDED.

We propose to continue study of the glycoprotein hormone. <u>erythropoietin</u>. Purification methods will be improved with the use of <u>affinity</u>, <u>chromatography</u> based on the newly developed <u>monoclonal</u>, <u>antibody</u>. We plan to prepare different hybridomas as a means of finding <u>antibodies</u> directed against different domains. We will continue the study of erythropoietin primary structure, both of the protein and carbohydrate portions. The <u>chemical</u> and <u>biological</u> properties of fragments of erythropoietin found in sera and urine will be determined. We will continue to search for a system in which to study the <u>biogenesis</u> of erythropoietin and its regulation. An improved radioimmunoassay, based on the use of the monoclonal antibody, will also be developed. radioimmunoassay, based on the use of the monoclonal antibody, will also be developed.

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LABORATORY ANIMALS INVOLVED. Identify by common names. If name, state "name"

Yes, mice, rats, rabbits.

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PAGE 3

CONFIDENTIAL SUBJECT TO COURT PROTECTIVE GRDER SECTION 1.

PAGE NUMBERS

| PRINCIPAL | INVESTIGAT | IND/PONCDAL | 4 DIRECTOR: | Eugene | Goldwasser |
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TABLE OF CONTENTS

Number pages consecutively at the bottom throughout the application. Do not use suffixes such as 5e, 5b. Type the name of the Principal Investigator/Program Director at the top of each printed page and each continuation page.

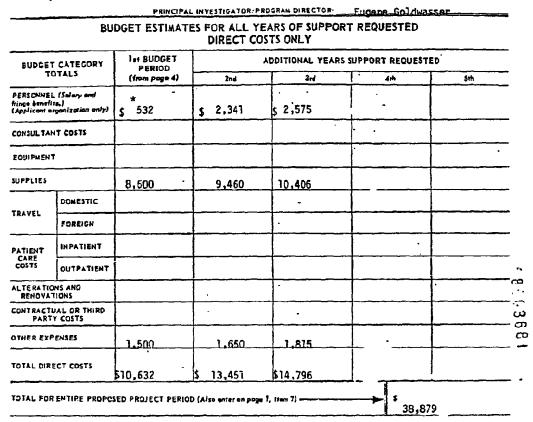
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Application Receipt Record, form PHS 3830 Form HHS 596 if Item 4, page 1, is checked

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Justification:

The 2.5 hours of an instrument designer's time are included in this project because the machine shop is involved in a) repair and maintenance of equipment not covered by maintenance contracts and b) construction of laboratory items not available from commercial sources, such as microelectrophoresis apparatus, which have

become vital to the research in progress.

This request for supplemental funds is justifiable by the greatly increased cost of contemporary research. Our work on the structural properties of erythropoietin and the biological and clinical aspects of its activity would be materially impeded by not being able to use state-of-the-art methods such as HPLC, affinity chromatography and gas chromatography. Our experience over the past two years makes it quite clear that even with great care in regulating expenditures, the amount available to us is inadequate. We have managed to make good progress because funds were made available that were, otherwise, in a restricted category, but it would be preferable to have the flexibility inherent in no restrictions.

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PAGE 1

SUBJECT TO COURT PROTECTIVE ORDER

Name of PI/PC/Program Coordinator or Candidate (Last, first mitial)

Goldwasser, Eugene

494-14-6535

* The wages for the instrument designer are calculated for the 3 months, 1 April 1983 to 30 June 1983 in the 1st year. After that the full year is used. Cost are increased by 10% per year. No change in percent effort of P.I.is involved.

I have included a modest amount (\$1500) for maintenance contracts which is intended to supplement that already committed to keeping sophisticated instruments in operation. The most critical maintenance contracts are for the gamma counters, the liquid scintillation counter and the high-speed centrifuges. These are items of equipment in constant use and downtime would impede our work. These contracts (also paid for by another grant) include preventive maintenance which does help keep the operation going. Because of the excessive expense we do not have maintenance contracts on the gas chromatograph or the HPLC; this is a calculated risk.

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PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: Eugene Goldwasser BIOGRAPHICAL SKETCH Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person. BIRTHDATE (Ma., Day, YL.) HAME Professor of Biochemistry 10-14-22 . Eugene Goldwasser EDUCATION (Begin with bacceleureure training and include postdoctoral) FIELD OF STUDY DEGREE INSTITUTION AND LOCATION CONFERRED The University of Chicago, Chicago, IL S.B. 1943 Biochemistry 1950 The University of Chicago, Chicago, IL Ph.D. Biochemistry RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with process position, list in chronological order provious employment, experience, and honors, include present membership on any Foderal Government Public Advisory Committee. List, in chronological order, the titles and gamplete references to recent representative publications, aspecially those most portinent to this application. Do not exceed 2 pages. Positions: Research Associate: Department of Biochemistry, University of Chicago 1952 - 1961 1962 - 1963 1963 - present Associate Professor of Biochemistry: University of Chicago Professor of Biochemistry, University of Chicago Chairman, Committee on Developmental Biology, University of Chicago 1976 - present Guggenhein Fellowship Oxford University, U.K. 1966 - 1967 Publications: (Selected from 109 papers since 1947): Meints, R., and Goldwasser, E. The persistence of Hemopoletic stem cells in vitro J, Cell Biol. 56: 429 (1973). Chang, C.S., and Goldwasser, E. On the mechanism of erythropoietin-induced differentiation XIII. A cytoplasmic protein mediating induced nuclear KNA synthesis. Dev. Biol. 34: 246 (1973). Chang, S.C.-S., Sikkema, D., and Goldwasser, E. Evidence for an erythropoletin receptor protein on rat bone marrow cells. Biochem. Biophys. Res. Commun. 57: 399 (1974). Goldwasser, E., Kung, C.K.-H., and Eliason, J.F. On the mechanism of erythropoietin-induced differentiation XIV, the role of sialic acid in erythropoietin action. J. Biol. Chem. 249: 4202 (1974). Goldwasser, E., Eliason, J.F., and Sikkema, D. An assay for erythropoletin in vitro at the milliunit level. Endocrinology 97: 315 (1975). Goldwasser, E. Erythropoietin and the differentiation of red blood cells. Fed. Proc. 34: 2285 (1975). Bedard, D.L., and Goldwasser, E. On the mechanism of erythropoietin-induced differentiation. XV. Induced transcription restricted by cytosine arabinoside. Exp. Cell Res. 102: 376 (1976) CO Van Zant, C., and Goldwasser, E. The effects of erythropoietin in vitro on spleen colony-forming cells. J. Cell Physiol. 90: 241 (1977). Miyake. T., Kung, C.K.-H., and Goldwasser, E. Purification of human erythropoietin. J. Biol. Chem. 252: 5558 (1977). دن C Van Zaut, C., and Coldwasser, E. The simultaneous effects of erythropoietim and ∞ colony stimulating factor on bone marrow cells. Scinece 198: 733 (1977). Sherwood, J.B., and Goldwasser, E. Extraction of erythropoietin from normal kidneys. Endocrinology 103: 866 (1978). PHS-39E PAGE Rev. 10.79 A 196324 CONFIDENTIAL

Called a Links of the party of 494-14-6535 Publications (contt) Goldwasser, E., and Inana, G. Molecular aspects of the initiation of erythropolesis in homopoietic cell differentiation. Eds. D.W. Golde, M.J. Cline, D. Hetcalf, and C.F. Fox, pp. 15-28, Academic Press, 1978. Van Zant. G., and Goldwasser, E. Suppression of erythroid differentiation by colony stimulating factor. Exp. Hematol, Today, eds. S.J. Baum & G.D. Ledney, pp63-71, Springer-Verlag, New York Inc. Van Zant, G., and Goldwasser, E. Competition between erythropoletin and colonystimulating factor for target cells in mouse marrow. Blood 53: 946 (1978). Elizson, J.F., Van Zant, G., and Goldwasser, E. The relationship of hemoglobin synthesis to erythroid colony and burst formation. Blood 53: 935 (1979): Sherwood, J.B., and Goldwasser, E. Radioimmunoassay for erythropoietin. Blood 54:885 (1979). Elizson, J.F., and Goldwasser, E. Evidence for cellular cooperativity in hemoglobin synthesis by erythroid bursts. Exp. Hematol. 8: 419 (1980).
Terasawa, T., Ogawa, M., Porter, P.N., Golde, D.W. and Goldwasser, E. Effect of burst-promoting activity (BPA) and erythropoietin or hemoglobin biosynthesis in culture. Blood 56, 7105 (1980). SITTLE-UINVIND FRANCIN Koeffler, H.P. and Goldwasser, E. Erythropoietin radioimmunoassay in evaluating patients with polycythemia. Ann. Int. Med. 94: 44 (1981). Weiss, TL and Goldwasser E. The biological properties of endotoxin-free human erythro-poietin. Biochem J 98, 17 (1981)

Goldwasser E. Erythropoietin and red cell differentiation in Control of Cellular Division and Development p 487 Eds D Cunningham, E Goldwasser, D Watson and CF Goldwasser E and Sherwood JB Radioimmunoassay of erythropoietin. Brit J Haematol 98, 359 (1981) Tong BD and Goldwasser E. The formation of erythrocyte membrane proteins during erythropoietin-induced differentiation. J Biol Chem 256, 12656(1981)
Nijhof W, Wierenga P and Goldwasser E. The regeneration of stem cells after a bone marrow depression induced by thiamphenicol. Exp Hematol 10, 36-43 (1982) 200 Ξ 11.1 2 A 196325 CONFIDENTIAL

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: EUGENE GOTOWASSET

OTHER SUPPORT (USE CONTINUATION PAGES IF HECESSARY)

For each of the professionals named on page 2, list, in three separate groups: (1) active support; (2) applications pending review and/or funding; (3) applications planned or being prepared for submission. Include all Federal, non-Federal, and institutional grant and contract support. If name, state "NONE." For each item give the source of support, identifying number, project title, name of principal investigator/program director, time or percent of effort on the project by professional named, annual direct costs, and entire period of support. (If part of a larger project, provide the titles of both the parent grant and the subproject and give the annual direct costs for each.) Briefly describe the contents of each item listed. If any of these avertop, duplicate, or are being replaced or supplemented by the present application, justify and delineate the nature and extent of the scientific and budgetary overlaps or boundaries.

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: (1) ACTIVE SUPPORT:

1. NIH: Grant HL 21676-05 Erythropoietin, Purification, Properties, Biogenesis, P.I. Eugene Goldwasser(15%), annual direct costs 07/01/81-06/30/82, \$153,751, period of support 07/01/77 to 06/30/85. Dr. F. F. Wang does not have any independent research support. She devotes 100% of her time to HL 21676.

NIH: Grant CA 18375 Hemopoietic Stem Cells and Induced Differentiation, P.I. Eugene Goldwasser (20%), annual direct costs 07/01/81 to 06/30/82 \$75,154, period of support 07/01/78 to 06/30/83. This project is devoted to the cell biology of erythropoietin and the relationships between erythropoietin-responsive cells and pluripotent stem cells.

NIH: Grant HL 16005, Comprehensive Center for Sickle Cell Research, project period 04/01/74 to 03/31/83, P.I. J. E. Bowman: Subproject; studies of erythropoiesis in vitro, P.I. Eugene Goldwasser (10%) & M. Gross, annual direct cost 05/01/82 to 04/31/83, \$89,724 (approximately one-half of this amount is for Dr. Gross' lab). This project is devoted to study of transcription induced by erythropoietin, to the regulation of heme synthesis and to the specific expression of mouse globin genes.

- Application pending: NIH HL 21676-06, Erythropoietin, Purification, Properties, Biogenesis, annual direct cost 07/01/82 to 06/30/83, \$164,023, period of support, 07/01/77 to 06/30/85.
- 3. Application planned: Program project on Sickle Cell Biology

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| Clinical: When needed, the clinical Research Center can be made available. It can be used for any further clinical testing. Animal: Carlson Animal Research Facility is used to house all lab animals and to maintain them in a healthy state for experimental purposes. Computer: Office: There are separate offices for the P.I. and the secretary. | When needed, the clinical Research Center can be made available. It can be used for any further clinical testing. Animal: Carlson Animal Research Facility is used to house all lab animals and to maintain them in a healthy state for experimental purposes. Computer: Other: There are separate offices for the P.I. and the secretary. Other: | ent of availability to the project. Use "ather" to describe fecilities at other performance sites listed in Item 9, page 1, and et is for field studies. Using continuation pages if necessary, include a description of the nature of any collaboration with other | |
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Eugene Goldwasser

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR:

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

| NAME | TITLE | | BIRTHDATE (Ma., Dey, Yr.) |
|--------------------------|-----------------------------|----------------------|---------------------------|
| Fung-Fang, Wang | Research Asso | ciate . | 05/05/48 |
| EDUCATION (Begin with | h baccaloureate training on | d Include posidacion | al) |
| INSTITUTION AND LOCATION | DEGREE | YEAR CONFERRED | FIELD OF STUDY |
| National Taiwan (Univ.) | R.S | 1970 | Agricultural Chem. |
| Rutgers University | | 1971-73 | Biochemistry |
| Indiana University | Ph.D | 1973-77 | Riochemistry |
| | | | |

RESEARCH AND/OR PROPESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and henors. Include present membership on any Federal Gevernment Public Advisory Committee. List, in chronological order, the titles and exemplate enfarences to secont representative publications, aspecially those most partiment to this application. Do not exceed 2 pages,

Professional Experience:

Univ. of Chicago (1981-Present), Research Associate

1. Structural and Functional Studies of Colony stimulating factor
2. Structural studies of erythropoietin
Univ. of Chicago (1979-1980), Post Doctoral Trainee
Purification of human urinary colony stimulating factor
City of Hope Medical Center (1977-1978), Junior Research Scientist
1. Interaction of detergents with fibronectin
2. Protein sequence studies of fibronectin

 Protein sequence studies of fibronectin
 University of Chicago (1979-1980): Post doctoral trainee, purification of human urinary colony stimulating factor

City Hope National Medical Center (1977-78): Junior Research Scientist.

<u>Publications:</u>

Pietrusako, R and Chen FF. (1976) Biochem Pharmacol 25, 2721.
Wang FFC, and Hirs CHW, (1977) J Biol Chem 252, 8358, Influence of the Heterosaccharides in porcine pancreatic ribonuclease on the conformation and stability

 Wang FFC, and Hirs CHM, (1979) J Biol Chem 254, 1090. A comparison by 220 MHz NMR of HIstidine H ion titration in porcine ribonuclease and an extensively deglycosylated derivative.

Wang FFC and Goldwasser E. Purification of Human urinary CSF (in preparation).
 Wang FFC and Goldwasser E. Irrelevance of the carbohydrate moiety of human

urinary CSF for activity. In preparation.

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| PRINCIPAL | INVESTIGATOR | TROCRAM | DIRECTOR: |
|-----------|--------------|---------|-----------|

Eugene Goldwasser

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Pingram Director. Photocopy this page for each person.

| NAME | TITLE | | BIRTHDATE (Ma., Day, Yr.). |
|--------------------------|---------------------------|----------------------|----------------------------|
| Fung-Fang, Wang | Research Asso | ciate | 05/05/48 |
| EDUCATION (Begin with | baccaloureote training en | d include postdactor | el) |
| INSTITUTION AND LOCATION | DEGREÉ | YEAR CONFERRED | FIELD OF STUDY |
| National Taiwan (Univ.) | B.5 | 1970 | Agricultural Chem. |
| Rutgers University | | 1971-73 | Biochemistry |
| Indiana University | Ph D | 1973-77 | Biochemistry |

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order provious amplayment, experience, and honors, include present membership on any Federal Government Public Advisory Committee, List, in chronological order, the titles and camplete references to recent representative publications, aspecially those most partinent to this application. Do not unever 2 pages.

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