EXHIBIT 5

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FORM APPROVED
O.M.B. NO. 68-R0249

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE

GRANT APPLICATION

FOLLOW INSTRUCTIONS CAREFULLY

LEAVE BLANK

TYPE	ACTIVITY	NUMBER
REVIEW GROUP		FORMERLY
COUNCIL/BOARD (Month, year)		DATE RECEIVED

1. TITLE OF APPLICATION (Do not exceed 56 typewriter spaces)
Erythropoietin: Purification, Properties, Biogenesis Supplement

2. RESPONSE TO SPECIFIC PROGRAM ANNOUNCEMENT [x] NO [ ] YES (If "YES," state RFA number and/or announcement title)
4-1-83 - 6-30-83

3. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR

3a. NAME (Last, first, middle)
Goldwasser, Eugene

3b. SOCIAL SECURITY NUMBER
494-14-6535

3c. MAILING ADDRESS (Street, city, state, zip code)
The University of Chicago
920 East 58th Street
Chicago, Illinois 60637

3d. POSITION TITLE
Professor

3e. DEPARTMENT, SERVICE, LABORATORY OR EQUIVALENT
Biochemistry

3f. TELEPHONE (Area code, number and extension)
(312) 753-4911

3g. MAJOR SUBDIVISION
Biological Sciences Division

4. HUMAN SUBJECTS, DERIVED MATERIALS OR DATA INVOLVED
[ ] NO [x] YES (If "YES," form HHS 596 required)

5. RECOMBINANT DNA RESEARCH SUBJECT TO NIH GUIDELINES
[x] NO [ ] YES

6. DATES OF ENTIRE PROPOSED PROJECT PERIOD (This application)
From: 04/01/83 Through: 06/30/85

7. TOTAL DIRECT COSTS REQUESTED FOR PROJECT PERIOD (from page 5)
$ 38,879

8. DIRECT COSTS REQUESTED FOR FIRST 12-MONTH BUDGET PERIOD (from page 4)
$ 10,632

9. PERFORMANCE SITES (Organizations and addresses)
The University of Chicago
920 East 58th Street
Chicago, Illinois 60637

10. INVENTIONS (Competing continuation application only)
Were any inventions conceived or reduced to practice during the course of the project?
[ ] NO [ ] YES - Previously reported
[ ] YES - Not previously reported

11. APPLICANT ORGANIZATION (Name, address, and congressional district)
The University of Chicago
5801 South Ellis Avenue
Chicago, Illinois 60637
First Congressional District

12. ORGANIZATIONAL COMPONENT TO RECEIVE CREDIT FOR INSTITUTIONAL GRANT (See instructions)
Code [0][1] Description: School of Medicine

13. ENTITY IDENTIFICATION NUMBER
1362177139A1

14. TYPE OF ORGANIZATION (See instructions)
[x] Private Nonprofit
[ ] Public (Specify Federal, State, Local):

15. OFFICIAL IN BUSINESS OFFICE TO BE NOTIFIED IF AN AWARD IS MADE (Name, title, address and telephone number)
Donald S. Sigal, Director, Office of Sponsored Programs
5801 South Ellis Avenue
Chicago, Illinois 60637
312-962-8604

16. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION (Name, title, address and telephone number)
1)
2) Donald S. Sigal, Director, Office of Sponsored Programs (312) 962-8604

17. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

SIGNATURE OF PERSON NAMED IN 3a (In ink. "Per" signature not acceptable)
Eugene Goldwasser

DATE
8 April 1982

18. CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true and complete to the best of my knowledge, and accept the obligation to comply with Public Health Service terms and conditions if a grant is awarded as the result of this application. A willfully false certification is a criminal offense. U.S. Code, Title 18, Section 1001.

SIGNATURE OF PERSON NAMED IN 16 (In ink. "Per" signature not acceptable)

DATE

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DEFENDANTS DEPOSITION EXHIBIT 87 10-13-99

TRIAL EXHIBIT OM 97-10814 WGY

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Trial Exhibit 251
97-10814-WGY

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE

ABSTRACT OF RESEARCH PLAN

LEAVE BLANK

PROJECT NUMBER

NAME AND ADDRESS OF APPLICANT ORGANIZATION (Same as Item 11, page 1)

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

TITLE OF APPLICATION (Same as Item 1, page 1)

Erythropoietin: Purification, Properties, Biogenesis

Name, Title and Department of all professional personnel engaged 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 long-term objectives, making reference to the scientific disciplines involved and the health-relatedness of the project. The abstract 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, erythropoietin. Purification methods will be improved with the use of affinity, chromatography based on the newly developed monoclonal, antibody. We plan to prepare different hybridomas as a means of finding antibodies directed against different domains. We will continue the study of erythropoietin primary structure, both of the protein and carbohydrate portions. The chemical and biological 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 biogenesis of erythropoietin and its regulation. An improved 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 none, state "none"

Yes, mice, rats, rabbits.

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PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: Eugene Goldwasser

TABLE OF CONTENTS

Number pages consecutively at the bottom throughout the application. Do not use suffixes such as 5a, 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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Other Biographical Sketches (*Not to exceed two pages for each*) 9
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SECTION 3. Appendix (*Six sets*) (*No page numbering necessary for Appendix*)

Number of publications: ______ Number of manuscripts: ______
Other items (list):

00.03679

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

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PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: Eugene Goldwasser

DETAILED BUDGET FOR FIRST 12 MONTH BUDGET PERIOD DIRECT COSTS ONLY

FROM 4/1/83 THROUGH 6/30/83

DOLLAR AMOUNT REQUESTED (Omit cents)

PERSONNEL (Applicant organization only) (See instructions) NAME	TITLE OF POSITION	TIME/EFFORT %	Hours per Week	SALARY	FRINGE BENEFITS	TOTALS
Eugene Goldwasser	Principal Investigator	15		-0-	-0-	-0-
Anthony Kittler	Instructment Designer		2.5	$450	$82	$532
Fung-Fang Wang	Research Assoc.	100		0	0	0
SUBTOTALS				$450	$82	$532

Category		Total
CONSULTANT COSTS (See instructions)		0
EQUIPMENT (Itemize)		0
SUPPLIES (Itemize by category)	HPLC columns and hardware, \$3000; spectral grade reagents, \$400; isotopes (^{14}C, ^{3}H, ^{125}I,), \$1500; culture media, \$700; fetal calf serum, \$800; disposable sterile culture ware, \$1400; antibodies and protein A, \$500; high purity gas for G-C, \$300.	$8,600
TRAVEL	DOMESTIC	0
	FOREIGN	0
PATIENT CARE COSTS	INPATIENT	0
	OUTPATIENT	0
ALTERATIONS AND RENOVATIONS (Itemize by category)		0
CONTRACTUAL OR THIRD PARTY COSTS (See instructions)		0
OTHER EXPENSES (Itemize by category)	Maintenance contracts, \$1500	$1,500
TOTAL DIRECT COSTS (Also enter on page 1, Item 8)		$ 10,632

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PAGE 4

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PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: Eugene Goldwasser

BUDGET ESTIMATES FOR ALL YEARS OF SUPPORT REQUESTED DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		1st BUDGET PERIOD (from page 4)	ADDITIONAL YEARS SUPPORT REQUESTED			
			2nd	3rd	4th	5th
PERSONNEL (Salary and fringe benefits.) (Applicant organization only)		* $ 532	$ 2,341	$ 2,575		
CONSULTANT COSTS						
EQUIPMENT						
SUPPLIES		8,600	9,460	10,406		
TRAVEL	DOMESTIC					
	FOREIGN					
PATIENT CARE COSTS	INPATIENT					
	OUTPATIENT					
ALTERATIONS AND RENOVATIONS						
CONTRACTUAL OR THIRD PARTY COSTS						
OTHER EXPENSES		1,500	1,650	1,815		
TOTAL DIRECT COSTS		$10,632	$ 13,451	$14,796		

TOTAL FOR ENTIRE PROPOSED PROJECT PERIOD (Also enter on page 1, Item 7) → $ 38,879

JUSTIFICATION (Use continuation pages if necessary): Briefly describe the specific functions of the personnel and consultants. For all years, justify any costs for which the need may not be obvious, such as equipment, foreign travel, alterations and renovations, and contractual or third party costs. For future years, justify any significant increases in any category. In addition, for COMPETING CONTINUATION applications, justify any significant increases over current level of support. If a recurring annual increase in personnel costs is anticipated, give percentage.

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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Name of PI/PD/Program Coordinator or Candidate (Last, first initial)	Social Security Number
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.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Eugene Goldwasser	Professor of Biochemistry	10-14-22

EDUCATION (Begin with baccalaureate training and include postdoctoral)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
The University of Chicago,Chicago, IL	S.B.	1943	Biochemistry
The University of Chicago, Chicago, IL	Ph.D.	1950	Biochemistry

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to recent representative publications, especially those most pertinent to this application. Do not exceed 2 pages.

Positions:

Research Associate: Department of Biochemistry, University of Chicago	1952 - 1961
Associate Professor of Biochemistry: University of Chicago	1962 - 1963
Professor of Biochemistry, University of Chicago	1963 - present
Chairman, Committee on Developmental Biology, University of Chicago	1976 - present

Honors

Guggenhein Fellowship Oxford University, U.K. 1966 - 1967

Publications: (Selected from 109 papers since 1947):

Meints, R., and Goldwasser, E. The persistence of Hemopoietic 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 RNA synthesis. Dev. Biol. 34: 246 (1973).

Chang, S.C.-S., Sikkema, D., and Goldwasser, E. Evidence for an erythropoietin 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 erythropoietin 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)

Van Zant, G., 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).

Van Zant, G., and Goldwasser, E. The simultaneous effects of erythropoietin 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).

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Continuation page 494-14-6535

Publications (con't)

Goldwasser, E., and Inana, G. Molecular aspects of the initiation of erythropoiesis in hemopoietic cell differentiation. Eds. D.W. Golde, M.J. Cline, D. Metcalf, 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 erythropoietin and colony-stimulating factor for target cells in mouse marrow. Blood 53: 946 (1979).

Eliason, 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).

Eliason, J.F., and Goldwasser, E. Evidence for cellular cooperativity in hemoglobin synthesis by erythroid bursts. Exp. Hematol. 8: 219 (1980).

Terasawa, T., Ogawa, M., Porter, P.N., Golde, D.W. and Goldwasser, E. Effect of burst-promoting activity (BPA) and erythropoietin on hemoglobin biosynthesis in culture. Blood 56, 1105 (1980).

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 erythropoietin. 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 Fox. 1981

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, 12666(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)

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PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: Eugene Goldwasser

OTHER SUPPORT
(USE CONTINUATION PAGES IF NECESSARY)

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 none, 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 overlap, 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.

2. 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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PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: Eugene Goldwasser

RESOURCES AND ENVIRONMENT

FACILITIES: Mark the facilities to be used and briefly indicate their capacities, pertinent capabilities, relative proximity and extent of availability to the project. Use "other" to describe facilities at other performance sites listed in Item 9, page 1, and at sites for field studies. Using continuation pages if necessary, include a description of the nature of any collaboration with other organizations and provide further information in the RESEARCH PLAN.

[X] Laboratory: Approx. 1500 sq. ft: fully operating, including culture labs, and needed equipment

[X] Clinical: When needed, the clinical Research Center can be made available. It can be used for any further clinical testing.

[X] 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.

[ ] Other (____________):

8-3686

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location, and pertinent capabilities of each.

HPLC, Gas chromatograph, culture hoods, incubators, centrifuges, monitors, counters and spectrophotometers are all within the lab.

ADDITIONAL INFORMATION: Provide any other information describing the environment for the project. Identify support services such as consultants, secretarial, machine shop, and electronics shop, and the extent to which they will be available to the project.

Secretarial service within the lab; the machine shop is an important adjunct as noted in the budget justification.

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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.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Fung-Fang, Wang	Research Associate	05/05/48

EDUCATION (Begin with baccalaureate training and include postdoctoral)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
National Taiwan (Univ.)	B.S.	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 previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to recent representative publications, especially those most pertinent 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

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.

Publications:

1. Pietrusako, R and Chen FF. (1976) Biochem Pharmacol 25, 2721.
2. Wang FFC, and Hirs CHW, (1977) J Biol Chem 252, 8358, Influence of the Hetero-saccharides in porcine pancreatic ribonuclease on the conformation and stability of the protein.
3. Wang FFC, and Hirs CHW, (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.
4. Wang FFC and Goldwasser E. Purification of Human urinary CSF (in preparation).
5. Wang FFC and Goldwasser E. Irrelevance of the carbohydrate moiety of human urinary CSF for activity. In preparation.

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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.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Fung-Fang, Wang	Research Associate	05/05/48

EDUCATION (Begin with baccalaureate training and include postdoctoral)

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
National Taiwan (Univ.)	B.S.	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 previous employment, experiences, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to recent representative publications, especially those most pertinent to this application. Do not exceed 2 pages.

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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

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.

Publications:
1. Pietrusako, R and Chen FF. (1976) Biochem Pharmacol 25, 2721.
2. Wang FFC, and Hirs CHW, (1977) J Biol Chem 252, 8358, Influence of the Hetero-saccharides in porcine pancreatic ribonuclease on the conformation and stability of the protein.
3. Wang FFC, and Hirs CHW, (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.
4. Wang FFC and Goldwasser E. Purification of Human urinary CSF (in preparation).
5. Wang FFC and Goldwasser E. Irrelevance of the carbohydrate moiety of human urinary CSF for activity. In preparation.

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Name of PI/PD/Program Coordinator or Candidate (Last, first initial)	Social Security Number
Goldwasser, Eugene	494-14-6535

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Objective: We plan to prepare enough pure human erythropoietin (epo) to: a) finish the determination of the primary structure of the polypeptide chain, b) start on an enzymic method of determining the sequence of the sugars in the oligosaccharide chains once we have methods for their separation, c) study the structural requirements for the biological activity of epo and d) continue the study of its clinical efficacy and physiological properties in vivo. In addition, we will try to develop additional monoclonal antibodies to epo with the intent of studying different domains against which the antibodies may be directed. The existence of naturally occurring immunoreactive fragments of epo in serum and urine makes it possible to isolate them in pure state and use them, as well, for additional studies of biological and immunological activities.

Review: Since the last competitive renewal of this grant there has been substantial progress in in this field. With the use of pure epo, (1) supplied from this laboratory, radioimmunoassay (RIA) was made possible (2,3) and is now operating on a fairly routine basis in three other institutions. The results with the RIA have largely confirmed the basic physiology of epo as determined by more cumbersome and less sensitive bioassays. (2,4). Additionally the RIA has permitted the determination of the normal serum titer of epo (5) and has been shown to provide a means of distinguishing between primary and secondary polycythemia (6). The existence of immunoreactive fragments of epo, presumably without biological activity, in sera of patients with chronic renal disease has been shown in two laboratories (3,7). Success in developing a hybridoma that forms a monoclonal anti-epo was reported from two laboratories (8,9). One of these antibodies is non-neutralizing, the others appear to be neutralizing.

There have been several reports of assays for epo by cultured cells (10-15), generally based on the original observations made in this lab in 1963 (16). There have also been several studies of circulating epo in disease states, for the most part using bioassay methods (17-21). A comparison between the bioassay and the hemagglutination-inhibition method showed the latter to be wanting (22). There have been several important additions to techniques for purifying epo reported (23-27). Two short papers on some chemical properties of epo in the crude state have appeared (28-29), and two on epo producing tumor cells (30,31). Schooley showed that an $F(ab')_2$ fragment derived from a neutralizing anti-epo, formed a complex with epo that was biologically active (32).

Progress report: In the three years since this project was last reviewed progress has been, in my view, substantial. With respect to the chemistry of epo we now know that the assumption of purity, based on chromatography and gel electrophoresis, was a valid one. In collaboration with Dr. Leroy Hood (California Institute of Technology) we have started the study of the amino acid sequence of both the α and β forms epo using the micro-solid-state sequencing method developed there. We find that both α and β epo have a single N terminal residue, alanine, and that the sequences of the next 27 residues are identical for the two forms. This suggests that the protein portions of the two may be identical, although differences based on amidation have not yet been ruled out.

Because of discrepancies in the amounts of carbohydrate and protein found by analytical techniques, we reinvestigated the molecular weight of epo. The previously reported value, 39000, was based on a sedimentation coefficient(s) of 3 S and a Stokes' radius ($\bar{r}$) of 32.5 Å. This value for M agreed with that found by gel electrophoresis in the presence of dodecylsulfate and mercaptoethanol. Since the value by electrophoresis seemed to be independent of gel concentration (over a rather narrow range) we provisionally accepted the value of 39 K. The value of M derived from s and $\bar{r}$ is dependent on the partial specific volume and using a tentative value of 40% for the carbohydrate content a value of $\bar{v}$=0.67 can be calculated. This results in M=34000 which is fair agreement with that found by the sum of the protein and carbohydrate contents.

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Social Security Number: 494-14-6535

We have made reasonable progress in our study of the carbohydrate composition of epo, but have not yet, because of the dearth of material, established the number of oligosaccharide chains. By scaling down the gas-liquid chromatographic method for determination of trifluoroacetyl derivatives of the monosaccharides, to permit quantitative determination of about 1 nmole, we have determined the carbohydrate composition of the α and β forms and found them to be significantly differently with respect to sialic acid and N-acetylglucosamine.

MONOSACCHARIDE COMPOSITION OF ERYTHROPOIETIN(residues/mole)

	α	β
Fucose	4.3	3.8
Galactose	11.7	10.1
Mannose	6.8	7.1
Glucose	0.7	0.7
N-acetylglucosamine	11.8	8.1
Sialic Acid	15.8	11.5
N-acetylgalactosamine	0	0

In addition, we have used two methods of removing carbohydrate from the glycoprotein: solvolysis with pyridine in anhydrous HF and by use of a preparation of mixed glycosidases (generously provided by Dr. R. Hill). In both cases, approximately 80% of the carbohydrate was removed; the only remaining sugar being N-acetylglucosamine attached to the protein. Biological activity, to the extent of about 80% was retained by the aglycoepo, as measured by the in vitro method. We have not yet done the in vivo assay. These findings suggest, strongly, that the carbohydrate is not required for the interaction of epo with its target cells, even though it might be required for in vivo "survival." They also make our longer range goal of cloning the epo gene and expressing its synthesis by bacterial cells a feasible one.

Over the past few years, since we found that labeling epo with ^{125}I either on the tyrosine residues on the free amino groups caused inactivation of its biological activity, we have been attempting to use the sulfhydryl groups of epo as a functionality for alkylalation with an iodinatable reagent. (The tritiation of epo on the carbohydrate is feasible and yields an active derivative but of too low specific activity to use for binding studies). The basis for our experiments was our finding that N-ethylmaleimide (NEM) reacts with epo to the extend of 3 moles/mole without loss of biological activity. This observation, it now appears, was artifactual; the NEM was not covalently bound. Our more recent data now clearly show that the SH groups are not accessible to the reagent unless epo is denatured and reduced. Under such conditions it reacts with NEM (4 moles/mole). The S-alkylated epo is devoid of biological activity, suggesting that two disulfide bonds are required. These findings make it apparent that our attempts to label with ^{125}I via the cysteine residues were futile.

There was, however, an interesting paradox revealed. The fluorescent SH reagent. (N-dimethylamino-4-coumarinyl)-maleimide reacts with epo to generate a derivative that is fluorescent and has full biological activity. Despite the fact that the product is not the epo thioether we first assumed, and even though its chemical nature is not at all clear, we have used it to study epo responsive cells. The frequency of these cells in marrow; about 1.4%,is the same using the unknown epo derivative as it is using biotin-labeled monoclonal anti-epo and fluorescein-labeled avidin.

As just indicated, we have succeeded in developing a hybridoma that forms monoclonal anti-epo. We screened about 3000 rat-mouse hybrids before we found one stable clone. The screening was done by a binding assay using ^{125}I-epo; the resulting antibody does not neutralize the biological activity. We have begun to accumulate purified monoclonal anti-epo IgG from the peritoneal ascites fluid of nude mice

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carrying the hybridoma and to develop an immunoaffinity method to purify epo. In regard to other epo purification techniques we have made a significant, if small, change in the published method that improves the yield considerably. The chromatographic step on sulfopropylsephadex in which we formerly had a yield of only 50% was improved by adding ethylene glycol to eluting buffer, to a final concentration of 20%. Under these conditions, the yield is 100%.

The use of the RIA and gel permeation chromatography permitted us to show that, in sera from patients with chronic renal disease, the most probable explanation for the discrepancy in epo titer between the RIA and the bioassay is the presence of immunoreactive "fragments" smaller than cytochrome-C. We find a similar situation in following the clearance of epo injected into patients on dialysis. There is a rapid loss of RIA titer from the circulation, followed by a secondary rise, in all three patients studied. Analysis of the molecular size of the immunoreactive material showed that in the rapid disappearance phase of the clearance curve there was about 75% native-sized epo and 25% as small fragments; in the secondary rise part of the curve there was about 25% native and 75% fragments. These data indicate that epo, in patients with chronic renal disease, is broken down after it is cleared and the breakdown product is released back into the circulation. We have also found evidence for small immunoreactive fragments in urine from patients with aplastic anemia.

Lastly, because of the great need for material for research in this field, we have supplied to NIH for distribution, two batches of epo. The first, for RIA purposes, is pure α form at 70,000 units/mg protein and we have given 95 vials at 57 U/vial to be allocated by a subcommittee. The second preparation is only 1100 U/mg of protein but has been freed of colony-stimulating factor, endotoxin and burst-promoting activity. It is non-inhibitory for mouse burst cultures up to 10 U/ml and also supports the growth of human bursts. We have provided 305,660 units, in aliquots of 1700 units, for distribution.

<u>Publications</u>:

JF Garcia, JB Sherwood and E Goldwasser Radioimmunoassay of erythropoietin. Blood Cells 5, 405-419 (1979)

JB Sherwood and E Goldwasser A radioimmunoassay for erythropoietin. Blood 54, 885-893 (1979)

T Terasawa, M Ogawa, PN Porter, DW Golde and E Goldwasser Effect of burst-promoting activity (BPA) and erythropoietin on hemoglobin biosynthesis in culture. Blood 56, 1106-1110 (1982)

HP Koeffler and E Goldwasser Erythropoietin radioimmunassay in evaluating patients with polycythemia. Ann Int Med 94, 44-47, (1981)

TL Weiss and E Goldwasser The biological properties of endotoxin-free human erythropoietin. Biochem J 198, 17-21 (1981)

E Goldwasser Erythropoietin and red cell differentiation in Control of Cellular Divsiion and Development 1981 pp 487-494 Eds. D Cunningham, E Goldwasser, D Watson, CF Fox, (AR Liss, NY)

BD Tong and E Goldwasser The formation of erythrocyte membrane proteins during erythropoietin induced differentiation. J Biol Chem 256, 12666-12672 (1981)

<u>In press</u>

CW Distelhorst, DS Wagner, E Goldwasser and JW Adamson Autosomal dominant familial erythrocytosis due to automomous erythropoietin production.

TL Weiss, CJ Kavinsky and E Goldwasser Characterization of a monoclonal antibody to human erythropoietin.

<u>Submitted</u>

JB Sherwood and E Goldwasser The heterogeneity of immunoreactive human serum erythropoietin.

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Social Security Number 494-14-6535

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Methods:

Because this is a request for a supplement, this section of the proposal will be in less detail than would be the case for a full grant.

By using the monoclonal antibody affinity column, already tested, and one or two other steps we plan to prepare as much pure epo as possible from the remaining material we have in stock. Now that the supply from Japan is not reliable, we would also be glad to arrange a cooperative mechanism by which we return one-half of the final yield of pure epo to a lab that provides a source of active urine. We estimate that the material now on hand is sufficient for about 5-6 years of the research discussed below, so we will not be hampered by scarcity. This state is aided by our success in improving the yield at the sulfopropyl Sephadex step.

In our continued search for alternative sources of human epo, we will use the affinity column to concentrate the epo in fractions of plasma prepared by the Red Cross. This is some indication that there may be a significant, small amount in one of the fractions that is routinely discarded. If we can find a simple method of recovering these small amounts, the many thousands of liters of plasma may be a secondary source.

We will continue our collaboration with Prof. Hood in order to get as much sequence information as possible. This sequence will then be used in several different types of research: for studies of structure-function relationships, to prepare fragments of known structure by limited proteolysis (e.g. trypsin and chymotrypsin at very low concentration, short time and low temperature, V8 protease) or by chemical cleavage methods at specific residues, such as methonine, tryptophan and perhaps cysteine. In this last case we have already found that we can prepare such fragments, but since the N termini are blocked we cannot use them for sequencing. The fragments may be useful for study of specificity of interaction with antibodies, but that will have to wait until we have the sequence of the whole polypeptide so we know the structure of the fragments broken at the cysteines.

The sequence information will also be used to prepare a synthetic oligodeoxy nucleotide probe, following the method of Agarwal, for the eventual purpose of isolating the epo mRNA as an essential prerequisite to preparing the cDNA that can be cloned. This last aspect is still some time away.

We are also going to use pure epo labeled with ^{3}H in the terminal sialic acids, to isolate, by HPLC, the oligosaccharide chains, after pronase digestion of epo. Once this is accomplished, we will use stepwise hydrolysis by specific glycosidases for structure studies. After each enzyme is used we will determine the released monosaccharide, if any, by gas-liquid chromatography techniques already developed for the composition studies. This method involves "guessing" which glycosidase to use at which step but is still feasible, whereas the NMR technique used for orosomucoid would require much more epo than we can produce within the next several years.

As we continue to study the structure of human epo, we will use the affinity column method for the purification of rabbit epo. Over the past years we have accumulated many liters of active rabbit serum that are stored frozen, waiting for this method. If our monoclonal antibody does not react with rabbit epo (not yet tested) we will continue to produce similar antibodies until we find one that does cross react. There is reason to believe that such cross reaction will not be too difficult to find since we have tested a rabbit anti-human epo and found it to react with both sheep and mouse epo. Pure rabbit epo, once we convinced of its homogeneity, will be sequenced in order to determine whether there are homologous structural features in the two species and whether those features are involved in biological activity.

The presently available monoclonal antibody is non-neutralizing. We will prepare other antibodies, in the search for one that neutralizes, and to accumulate antibodies directed at as many different domains of the epo molecule as possible. The antibodies will be produced, either by the rat-mouse hybrid method that has already been successful or by <u>in vitro</u> immunization. The non-neutralizing antibody can

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be used to label epo for binding studies. We will prepare the Fab fragment, in order to avoid problems with Fc receptors, label it with ^{125}I, make the Fab-epo complex and use that to study quantitative binding by marrow and other potentially epo-responsive cells. Labeling with fluorescein and/or rhodamine will also be used in order to identify, by microscopy, the cells with epo receptors. This will be an important part of our effort in the immediate future. Among the questions that need exploration by these methods are: a) since CFU-E can be purified to a great extent, what is the number of receptors for epo on these late erythroid progenitors? b) how does that compare with the number per BFU-E? These latter cells cannot, yet, be purified to any great extent, but we can prepare marrow cell populations almost devoid of CFU-E by keeping mice plethoric for some time; the number of BFU-E is not appreciably changed. By counting the number (frequency) of labeled cells in such a population and by direct binding studies of labeled epo-Fab we can determine the mean number of binding sites per cell. These should represent pre CFU-E, including BFU-E; the latter can, of course, be estimated to a rough extent by burst counting. We also plan to test a variety of erythroid-like cell lines such as FLC, K562, and HEL for epo binding.

The monoclonal anti-epo will also be used to develop a solid-state RNA based on the specificity of the antibody, which can be "grown" in large amount rather than on the, still rare, antigen. This method, if the volumes and amounts can be adjusted, could be 4-5 x more sensitive than the existing RIA, especially since the label (^{125}I) will be on a commercially available reagent, protein A. We can thus use more label and more reagent to be labeled. We need a more sensitive (and would like a more rapid) assay, in order to study the fragments of epo found in serum and to determine whether fragmentation is a general, non-pathological, phenomenon, or restricted to certain disease states. Similarly a more sensitive assay will be of great help in the study of clearance rates in laboratory animals.

Although we have screened eight human renal tumors in the past year we have not yet found one that stably secretes epo in significant amounts; two of the eight did show a transient production of epo which decreased within a few days. None of the tumors was from a patient with erythrocytosis. We intend to continue this screening and, for those cells that can be established in culture to study the effects of methods known to increase epo production in vivo; these include hypoxia, colbalt, cyclic AmP, prostaglandin, testosterone and combinations of them. These tumor cells, if they can be shown to either produce epo in vitro or contain a large amount, can be used as sources of epo mRNA as indicated above.

Last to be mentioned in this brief account of our experimental aims, is the accumlation of enough pure epo to do a significant clinical trial of its effect in the correction of the anemia of chronic renal disease, and possibly of inflamatory diseases. Preliminary experiments, indicate that epo may well be useful in renal disease patients. When enough material is at hand we will continue these trials and when possible we will initiate collaborative studies in other institutions.

Justification for supplemental funds: The laboratory staff consists of two professional biochemsits, three technicians, a lab helper and two graduate students who are not being paid by this grant. The kinds of problems we are studying are expensive since they involve costly HPLC columns, ultra pure reagents and solvents, expensive animals, animal care and media for cell culturing. Despite our realization that funds are limited and our careful monitoring of each expenditure, we have found, in the last two years, that the non-personnel budget simply does not provide enough funds for our work. Some of the funds restricted, by the study section; for epo purchase were made available to us during the past two years for general purposes, because the Japanese source of epo is now very uncertain. This special lifting of restriction permitted us to carry out our experimentation for the full year. Without those funds we would have been in the position of being able to do nothing but read and write for the last 2-3 months of the budget year. In part this "shortfall" is due to increases

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in expenditure that were not calculable when the original proposal was submitted. These are due to mandatory raises for the non-professional personnel, to extra-ordinary increases in animal care costs and to the shift to important new, but expensive, techniques such as HPLC.

I realize that this is already a large grant, but the size is not going to be materially changed since the bulk of the funds (78%) is used for personnel costs. There is added to this request, a small addition to the personnel category, that of the instrument designer for 2.5 hours/week. This represents part of the cost to the department of maintaining the machine shop. We do make considerable use of the services of the machine shop to service equipment not under maintenance contract and to construct items that are not commercially available such as micro gel electrophoresis apparatus which we now use routinely.

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DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

PROTECTION OF HUMAN SUBJECTS
ASSURANCE/CERTIFICATION/DECLARATION

[X] ORIGINAL [ ] FOLLOWUP [ ] REVISION

[X] GRANT [ ] CONTRACT [ ] FELLOW [ ] OTHER

[ ] NEW [ ] RENEWAL [ ] CONTINUATION

APPLICATION IDENTIFICATION NUMBER *(If known)*

STATEMENT OF POLICY: Safeguarding the rights and welfare of subjects at risk in activities supported under grants and contracts from DHEW is primarily the responsibility of the institution which receives or is accountable to DHEW for the funds awarded for the support of the activity. In order to provide for the adequate discharge of this institutional responsibility, it is the policy of DHEW that no activity involving human subjects to be supported by DHEW grants or contracts shall be undertaken unless the Institutional Review Board has reviewed and approved such activity, and the institution has submitted to DHEW a certification of such review and approval, in accordance with the requirements of Public Law 93-348, as implemented by Part 46 of Title 45 of the Code of Federal Regulations, as amended.(45 CFR 46). Administration of the DHEW policy and regulation is the responsibility of the Office for Protection from Research Risks, National Institutes of Health, Bethesda, Md 20014.

1. TITLE OF PROPOSAL OR ACTIVITY

Erythropoietin: Purification, Properties, Biogenesis

2. PRINCIPAL INVESTIGATOR/ACTIVITY DIRECTOR/FELLOW

Eugene Goldwasser

3. DECLARATION THAT HUMAN SUBJECTS EITHER WOULD OR WOULD NOT BE INVOLVED

[ ] A. NO INDIVIDUALS WHO MIGHT BE CONSIDERED HUMAN SUBJECTS, INCLUDING THOSE FROM WHOM ORGANS, TISSUES, FLUIDS, OR OTHER MATERIALS WOULD BE DERIVED, OR WHO COULD BE IDENTIFIED BY PERSONAL DATA, WOULD BE INVOLVED IN THE PROPOSED ACTIVITY. (IF NO HUMAN SUBJECTS WOULD BE INVOLVED, CHECK THIS BOX AND PROCEED TO ITEM 7. PROPOSALS DETERMINED BY THE AGENCY TO INVOLVE HUMAN SUBJECTS WILL BE RETURNED.)

[X] B. HUMAN SUBJECTS WOULD BE INVOLVED IN THE PROPOSED ACTIVITY AS EITHER: [X] NONE OF THE FOLLOWING, OR INCLUDING: [ ] MINORS, [ ] FETUSES, [ ] ABORTUSES, [ ] PREGNANT WOMEN, [ ] PRISONERS, [ ] MENTALLY RETARDED, [ ] MENTALLY DISABLED. UNDER SECTION 6. COOPERATING INSTITUTIONS, ON REVERSE OF THIS FORM, GIVE NAME OF INSTITUTION AND NAME AND ADDRESS OF OFFICIAL(S) AUTHORIZING ACCESS TO ANY SUBJECTS IN FACILITIES NOT UNDER DIRECT CONTROL OF THE APPLICANT OR OFFERING INSTITUTION.

4. DECLARATION OF ASSURANCE STATUS/CERTIFICATION OF REVIEW

[ ] A. THIS INSTITUTION HAS NOT PREVIOUSLY FILED AN ASSURANCE AND ASSURANCE IMPLEMENTING PROCEDURES FOR THE PROTECTION OF HUMAN SUBJECTS WITH THE DHEW THAT APPLIES TO THIS APPLICATION OR ACTIVITY. ASSURANCE IS HEREBY GIVEN THAT THIS INSTITUTION WILL COMPLY WITH REQUIREMENTS OF *DHEW Regulation 45 CFR 46*, THAT IT HAS ESTABLISHED AN INSTITUTIONAL REVIEW BOARD FOR THE PROTECTION OF HUMAN SUBJECTS AND, WHEN REQUESTED, WILL SUBMIT TO DHEW DOCUMENTATION AND CERTIFICATION OF SUCH REVIEWS AND PROCEDURES AS MAY BE REQUIRED FOR IMPLEMENTATION OF THIS ASSURANCE FOR THE PROPOSED PROJECT OR ACTIVITY.

[X] B. THIS INSTITUTION HAS AN APPROVED GENERAL ASSURANCE (DHEW ASSURANCE NUMBER G1626) OR AN ACTIVE SPECIAL ASSURANCE FOR THIS ONGOING ACTIVITY, ON FILE WITH DHEW. THE SIGNER CERTIFIES THAT ALL ACTIVITIES IN THIS APPLICATION PROPOSING TO INVOLVE HUMAN SUBJECTS HAVE BEEN REVIEWED AND APPROVED BY THIS INSTITUTION'S INSTITUTIONAL REVIEW BOARD IN A CONVENED MEETING ON THE DATE OF 12/1/81 IN ACCORDANCE WITH THE REQUIREMENTS OF THE *Code of Federal Regulations on Protection of Human Subjects (45 CFR 46)*. THIS CERTIFICATION INCLUDES, WHEN APPLICABLE, REQUIREMENTS FOR CERTIFYING FDA STATUS FOR EACH INVESTIGATIONAL NEW DRUG TO BE USED (SEE REVERSE SIDE OF THIS FORM).

THE INSTITUTIONAL REVIEW BOARD HAS DETERMINED, AND THE INSTITUTIONAL OFFICIAL SIGNING BELOW CONCURS THAT:

EITHER [X] HUMAN SUBJECTS WILL NOT BE AT RISK; OR [ ] HUMAN SUBJECTS WILL BE AT RISK.

5. AND 6. SEE REVERSE SIDE

7. NAME AND ADDRESS OF INSTITUTION

The University of Chicago, 5801 South Ellis, Chicago, Illinois 60637

8. TITLE OF INSTITUTIONAL OFFICIAL	TELEPHONE NUMBER
SIGNATURE OF INSTITUTIONAL OFFICIAL	DATE

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HEW-596 (Rev. 4-75)

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PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: Eugene Goldwasser

CHECKLIST

This is the required last page of the application.

Check the appropriate boxes and provide the information requested.

TYPE OF APPLICATION:

☐ NEW application (*This application is being submitted to the PHS for the first time.*)

☐ COMPETING CONTINUATION of grant number: ______
(*This application is to extend a grant beyond its original project period.*)

☒ SUPPLEMENT to grant number: HL 21676
(*This application is for additional funds during a funded project period.*)

☐ REVISION of application number: ______
(*This application replaces a prior version of a new, competing continuation or supplemental application.*)

☐ Change of Principal Investigator/Program Director.
Name of former Principal Investigator/Program Director: ______

ASSURANCES IN CONNECTION WITH:

Civil Rights	Handicapped Individuals	Sex Discrimination	Human Subjects General Assurance (If applicable)	Laboratory Animals (If applicable)
☒ Filed	☒ Filed	☒ Filed	☒ Filed	☒ Filed
☐ Not filed	☐ Not filed	☐ Not filed	☐ Not filed	☐ Not filed

INDIRECT COSTS:

Indicate the applicant organization's most recent indirect cost rate established with the appropriate DHHS Regional Office. If the applicant organization is in the process of initially developing or renegotiating a rate, or has established a rate with another Federal agency, it should, immediately upon notification that an award will be made, develop a tentative indirect cost rate proposal based on its most recently completed fiscal year in accordance with the principles set forth in the pertinent DHHS Guide for Establishing Indirect Cost Rates, and submit it to the appropriate DHHS Regional Office. Indirect costs will not be paid on foreign grants, construction grants, and grants to individuals, and usually not on grants in support of conferences.

☒ DHHS Agreement Dated: 07/06/81
______ % Salary and Wages or 57 % ~~XXXXXXXXXX~~ MTDC

Is this an off-site or other special rate, or is more than one rate involved? ☐ YES ☒ NO
Explanation: ______

☐ DHHS Agreement being negotiated with ______ Regional Office.

☐ No DHHS Agreement, but rate established with ______ Date ______

☐ No Indirect Costs Requested.

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PROTECTION OF HUMAN SUBJECTS

Principal Investigator: Eugene Goldwasser

Department: Biochemistry

Title of Application: Erythropoietin: Purification, Properties, Biogenesis

Sponsoring Agency: NIH (External, Departmental, Other, Etc.)

(This form MUST be completed and submitted with all grant and contract applications, before processing in the Dean's Office. Additionally, one copy of this form must be submitted to the CIC when submitting any protocol involving Human Subjects for review.)

Check the following statements that pertain to your application:

1. ____ Human subjects are not included in this application.

2. __x__ Human Subjects Involved:

None of the following	(x)
Minors	( )
Fetuses	( )
Abortuses	( )
Pregnant Women	( )
Prisoners	( )
Mentally Retarded	( )
Mentally Disabled	( )

(cannot understand the proposed course of treatment)

3. __x__ Human subjects are involved in this application. The protocol has been reviewed and approved by our Clinical Investigation Committee.

   Date reviewed: 12/1/81 Protocol # 2399

4. ____ This research protocol was reviewed and approved by our Clinical Investigation Committee at the time I applied to another agency for funding:

   Specify other agency: ____

   Title of Application: ____

   Date reviewed: ____ Protocol # ____

5. ____ This application includes human subjects, but has not received approval by the Clinical Investigation Committee, and therefore, must be submitted. (This will be handled by the Dean's Office.)

6. ____ Do you intend to obtain informed consent in writing? Yes __x__ No ____

7. ____ If the informed consent is obtained in writing, will you devise a special form? Yes ____ No __x__. If the answer is yes, please enclose a copy of the intended statement.

8. ____ If research subjects are to be paid, please give us the details (budget page does not reach Clinical Investigation Committee.) Please indicate whether these subjects are patient volunteers or non-patient volunteers.

84103697

Eugene Goldwasser
Signature of Principal Investigator

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SUBJECT TO COURT PROTECTIVE ORDER

April 8, 1982
Date

Revised 9/77

A 196338
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EXHIBIT A

THE UNIVERSITY OF CHICAGO

STATEMENT TO ACCOMPANY APPLICATION FOR CONTRACT, GRANT OR AWARD

TO NIH
(Sponsoring Agency or Organization)

PROPOSAL TITLE:

Erythropoietin: Purification, Properties, Biogenesis

PRINCIPAL INVESTIGATOR(S): (Please type)

(1) Eugene Goldwasser

(2)

(3)

81 U3698

DATE: 4 / 7 / 82

The Principal Investigator(s) understand that any invention made or discovered by the Principal Investigator(s) or other staff in the course of activities encompassed by this application is subject to the terms of the University contract, grant or award document and rights shall be assigned and processed in accordance with the University Statute on patents now in effect. The Principal Investigator(s) agrees to ensure that all appropriate individuals working or consulting on this project shall be aware of this patent disclosure and assignment requirement.

Signed by Principal Investigator(s):

(1) Eugene Goldwasser

(2)

(3)

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BSD

RECOMBINANT DNA RESEARCH

Principal Investigator: Eugene Goldwasser

Department: Biochemistry

Title of Application: Erythropoietin: Purification, Properties, Biogenesis

Sponsoring Agency: NIH (External, Departmental, Other, Etc.)

(This form MUST be completed and submitted with all grant and contract applications, before processing in the Dean's Office. Additionally, one copy of this form must be submitted to the Institutional Biosafety Committee (IBC) if any recombinant DNA research is proposed in your application.)

Check the following statements that pertain to your application.

1. _x_ Experiments with recombinant DNA molecules[1] are not included in this application.

2. ___ Experiments with recombinant DNA molecules are included. According to the NIH Guidelines of January 1980, these experiments fall into one or more of the following categories:

(a) ___ Exempt from the Guidelines[2]. If so, provide the following information:

Nature of DNA sequences to be cloned ___

Source of DNA (organism) ___

Vector ___

Host ___

(b) ___ Governed by the Guidelines although an MUA need not be submitted[3]. If this is the case, provide the following information:

Nature of DNA sequence to be cloned ___

Source of DNA (organism) ___

Vector ___

Host ___

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(Note that this signed form containing the requested information serves as the required registration document.)

(c) ___ Governed by the Guidelines and requiring submission of an MUA[4]. If this is the case, an MUA must be prepared according to the format required by the IBC and the NIH Guidelines of January 1980 and submitted to the IBC Chairman for review and approval by the Committee.

NOTE THAT YOU MAY HAVE PROPOSED EXPERIMENTS IN ALL 3 OF THESE CATEGORIES.

April 8, 1982
Date

Eugene Goldwasser
Signature of Principal Investigator

CONFIDENTIAL SUBJECT TO COURT PROTECTIVE ORDER

NOTICE: See instructions on reverse of last copy.

FORM APPROVED
O.M.B. NO. 68-R0249

Prepared for the Science Information Exchange.
Not for publication or publication reference.

U. S. Department of
HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE

NOTICE OF RESEARCH PROJECT

PROJECT NO. (DO NOT USE THIS SPACE)

TITLE OF PROJECT

Erythropoietin: Purification, Properties, Biogenesis

GIVE NAMES, DEPARTMENTS, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS OR PROJECT DIRECTORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT.

Eugene Goldwasser, Department of Biochemistry Professor
Fung Wang, Department of Biochemistry, Research Professor

NAME AND ADDRESS OF APPLICANT INSTITUTION.

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

SUMMARY OF PROPOSED WORK—*(200 words or less) — Omit Confidential data.)*
In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research in the bio-sciences and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

We propose to continue to prepare and distribute pure human erythropoietin and to study possible improvements in fractionation methods. These improvements may include affinity chromatography using lectins and/or monoclonal anti erythropoietin, as well as high liquid chromatography. We will also study possible alternative large scale sources of erythropoietin, such as kidney extraction and cell culture methods. We will use the newly developed radioimmunoassay for screening. Successful erythropoietin production in cell culture may also permit study of its biogenesis and regulation. Improvement in the specificity of the radioimmunoassay will also be studied. We will continue to work on finding a method for radioiodination of erythropoietin with retention of biological activity, and to use such labeled material for the sutdy of physiological properties. Stimultaneously, we will continue the investigation of the chemical properties of erythropoietin with the intention of understanding the structural requirements for its biological activity, as a prerequisite for its eventual synthesis.

8003700

PROFESSIONAL SCHOOL *(medical, dental, etc.)* WITH WHICH THIS PROJECT SHOULD BE IDENTIFIED	SIGNATURE OF PRINCIPAL INVESTIGATOR	DATE
01 School of Medicine	[signature]	4/7/82

DO NOT WRITE BELOW THIS LINE — FOR OFFICE USE ONLY

SUPPORTING AGENCY

METHOD OF SUPPORT *(Check one)*

☐ Agency Staff (Intramural) ☐ Negotiated Contract ☐ Special Project Grant ☐ Research Grant ☐ Other (Specify)

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FUNDS OBLIGATED CURRENT F.Y.	NUMBER OF FUTURE YEARS TENTATIVELY ASSURED	BEGINNING DATE	ESTIMATED COMPLETION DATE

PHS 166
Rev. 5/80

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