EXHIBIT 1
Part 6 of 14

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5. b. References (Cont'd)

2. Van Stone, J.C. and Max, P.: The Effect of Erythropoietin on the Anemia of Uremia.

American Society of Nephrology (abstract - 11th Annual Meeting, 1978, p. 100A) (See attached copy).

3. Urabe, A., Chiba, S., Kosaka, K., Takaku, F.: Response of Uremic Bone marrow Cells to Erythropoietin In Vitro.

Scand. J. Haematol. 17:335-340, 1976.

4. Fisher, J.W.: Erythropoietin: Pharmacology, Biogenesis and Control of Production.

Pharmacological Reviews, 24(3): 459-508, 1972.

5c. Clinical Protocol

(See attached graph).

a. Patient population

1. Initial studies of H-EPO will be performed in 3 or 4 adult individuals with anemia of chronic renal failure who are hemodialyzed three times weekly and are hematologically stable. Patients of either sex with or without kidneys will be eligible. Ideal candidates will not have a red cell transfusion requirement or be taking androgens to stimulate erythropoiesis.

Patients will be evaluated prior to H-EPO administration to exclude other contributing causes of anemia, to permit correction of conditions which might preclude response to H-EPO (e.g. limiting nutrient(s)), and to carefully delineate the pre-treatment state of erythropoiesis.

b. Protocol

Baseline Studies will include:

1. Serial CBC, platelets, reticulocyte count and differential.
2. Stools for blood.
3. Bone marrow aspiration and biopsy to determine cellularity, status of iron stores, number of normoblasts/1000 nucleated marrow cells.
4. Serum folic acid, B_{12}, serum iron, TIBC, serum ferritin, Coombs' & T4/FTI.
5. Chromium^{51} red cell mass and blood volume.
6. Ferrokinetics - to include plasma iron turnover (PIT) and incorporation of Fe^{59} into red cells (% iron utilization).
7. EKG, Chest X-ray, SMA-17, prothrombin time and partial thromboplastin time.

8. Serum H-EPO level - by radioimmunoassay (technique available in Dr. Goldwasser's laboratory).

9. Urinary H-EPO determinations (in patients with urine output).

Patients on study will be hospitalized for intravenous infusions of H-EPO as described below. They will be housed in the Clinical Research Center at the University of Chicago Hospitals. All acute hospital facilities and personnel are available to deal with any untoward reaction.

H-EPO will be given by intermittent slow intravenous infusion of 520 units (in 1.9ml volume over 15-20 minutes) every 12 hours. (See attached sheet on pharmacokinetic considerations). This dose aims to raise the average serum H-EPO concentration to 50mu/ml (approximately 3x the average normal level of 16.5mu/ml). Patients with the anemia of chronic renal failure have serum H-EPO levels at (by bioassay) or slightly above (by radioimmunoassay) the normal in baseline conditions. Calculations of the pharmacokinetics are based upon the assumption of a plasma T1/2 of approximately 12 hours (ref. 4) lack of significant urinary loss in these patients and no loss by dialysis (molecular weight is too high). It is proposed to treat for 7 days (14 doses) with a subsequent observation period of 2 weeks.

Parameters to be monitored during and after the treatment period include:

1. CBC, platelet, reticulocytes every other day using smallest possible amounts of blood so as not to obscure any significant red cell mass increase.

2. Serum H-EPO levels daily with additional determinations after the first and last injections to evaluate disappearance time from plasma (radioimmunoassay is performed on samples of < 1ml serum).

3. Repeat SMA-17 on days 3, 7, and 21.

4. Repeat ferrokinetics and bone marrow studies - day 10.

5. Repeat Cr^{51} red cell mass and blood volume - day 21.

6. Daily 24 hr. urine collection for H-EPO assay (as available).

Pharmacokinetics (prepared by Dr. David Kornhauser - Department of Medicine; Section of Clinical Pharmacology; University of Chicago).

Assumed

1. First order kinetics - $C = C_o e^{-Kt}$

   At steady state - peak C^p and trough C^t concentrations are identical following every dose.

2. Time course: $C^t = C^p e^{-kT}$ where T=dosing interval

3. Average concentration (c) over dosing interval T = $\dfrac{\int_o^T C^p e^{-Kt}}{T}$

Average concentration (c) = $\dfrac{C^p e^{-Kt}}{KT} \Big/ {}_o^T$

$= \dfrac{C^p}{KT}\left(-e^{-Kt} \Big/ {}_o^T\right)$

$= \dfrac{C^p}{KT}\left(1-e^{-KT}\right)$

For T = 12 hr. = 1 T1/2
Dose = 520 units q 12 Hrs.

Average concentration (c) = $\dfrac{C^p}{KT1/2}\left(1-e^{-KT1/2}\right)$

$= \dfrac{C^p}{.693}\left(1-e^{.\frac{1}{693}}\right) = \dfrac{C^p}{.695}\left(1^{-}1/2\right)$

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$$\text{Avg. } C = \frac{C^p \cdot 1/2}{.693} =$$

$$\text{Avg. } C = .727C^p$$

4. For Avg. C of 50 mu/ml

C^p = 69.3 mu/ml

$C^t = \frac{C^p}{2}$ = 34.65 mu/ml

5. Expect 96.875% steady slate level at 60 hrs.

6. Distribution Space Estimate for Dose Calculations

a. For uremic patient: 70% Body Wt. = Water
ECF = 1/3 of total body water
70Kg subject
Space = (0.7)(.33)(70) ≃ 15 Liters

b. Clearance = K Vol.

K x t 1/2 = 0.693; t 1/2 = 12 hrs. (ref. 4)
K x 1/2 day = .693
K = 1.386/day

Clearance = 1.386 x 15,000 ≃ 20,800 ml/day

c. Clearance x Steady State Concentration = Infusion rate.

d. For steady State Concentration of 50 mu/ml
need 20,800 ml/day x 50 mu/ml =

1040 Units/day
to be infused

THE EFFECT OF ERYTHROPOIETIN ON THE ANEMIA OF UREMIA. John C. Van Stone and Paul Sax, Dept. of Medicine, Truman VAH and University of Missouri, Columbia, Missouri.

Although erythropoietin (EPO) is a potential therapeutic agent in anemia of uremia, previous studies suggest that there is resistance to its action in uremia which may limit its usefulness.

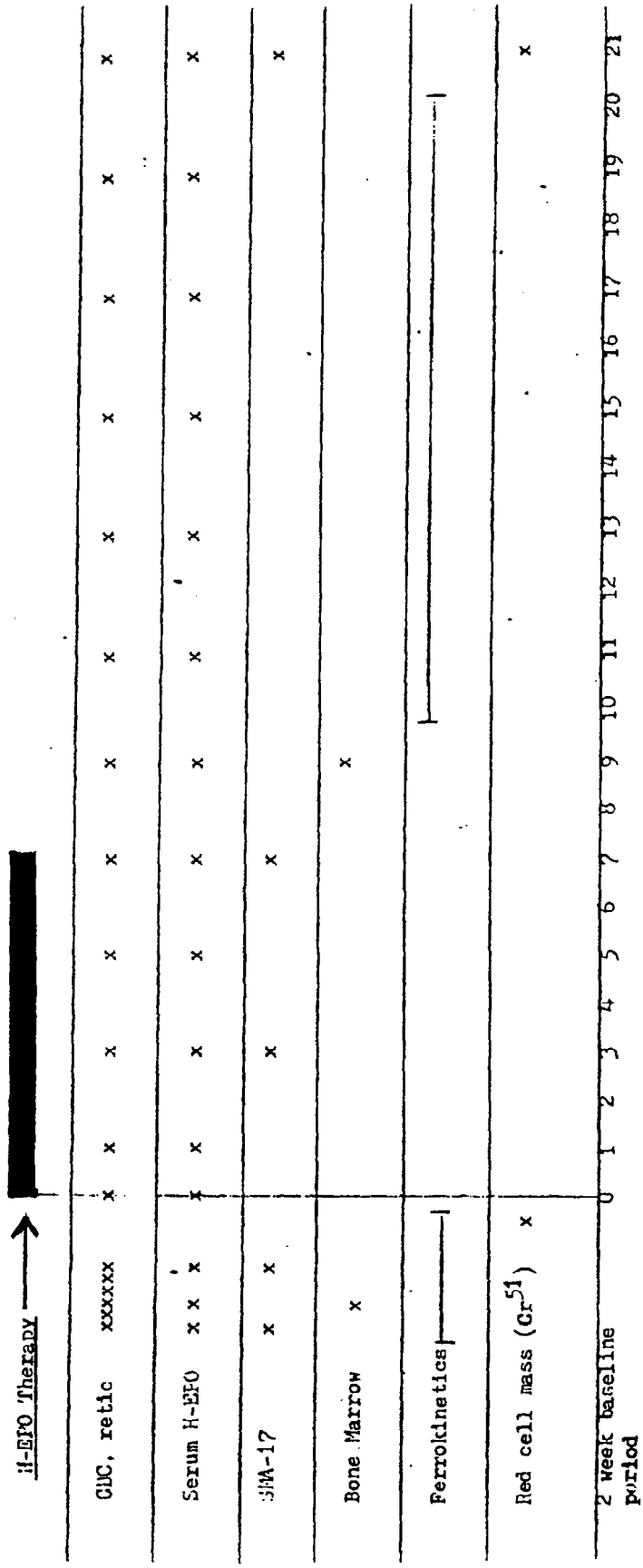
Four groups of 5 rats each were peritoneally dialyzed for 12 days: (1) Surgically anephric rats given 1 ml saline daily (NTX). (2) Anephric rats receiving 2 units of EPO/day (NTX+EPO). (3) Control sham operated saline infected rats (C). (4) Control rats given 2 units EPO (C+EPO). Plasma iron turnover (PIT) and red cell mass were determined after 8 days and RBC Fe^{59} uptake, marrow nucleated RBC, HCT and BUN determined at 12 days.

	NTX	NTX+EPO	C	C+EPO
BUN (mg/dl)	107$^{\phi}$	100$^{\phi}$	25	18
PIT (mg/kg/day)	1.01$^{\phi}$	1.84†	1.56	1.92
RBC Fe^{59} UPTAKE (%)	48$^{\phi}$	71†	74	76
NUCLEATED RBC/ FEMUR ($x10^7$)	0.64$^{\phi}$	2.52†	2.22	2.65
HCT (%)	19$^{\phi}$	30$^{\phi\dagger}$	42	48
RBC MASS (ml/kg)	14.0$^{\phi}$	21.4$^{\phi\dagger}$	24.0	31.4

$^{\phi}p < 0.05$ compared to C $^{\dagger}p < 0.05$ compared to NTX

Our data indicated that EPO completely corrects the marked bone marrow depression and significantly improves the anemia present in the uremic rat and suggests EPO will be a useful agent for the treatment of anemia of chronic renal failure.

CLINICAL PROTOCOL H-EPO



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6. Informed Patient Consent

Patient Name:

Protocol # 2822

Unit No.:

CONSENT BY SUBJECT OF RESEARCH PROJECT
(Short Form)

Research Project: Clinical Study of Purified Human Erythropoietin (H-EPO)

Doctor(s) Directing Project: Joseph Baron, M.D.; Dimitrios Emmanouel, M.D.; Eugene Goldwasser, Ph. and Adrian Katz, M.D.

I,_____, the undersigned, hereby consent to participate as a subject in the above named research project conducted by The University of Chicago Hospitals and Clinics.

I agree to receive H-EPO, a purified human hormone which is a new experimental drug, intravenously twice daily (at approximately 12-hour intervals) for 7 days as a test of its ability to stimulate blood formation in me. The preliminary and follow-up tests to evaluate its effects are standardized medical procedures. I agree to have periodic samples of blood and urine taken as part of the evaluation process. I understant that the purified hormone is of human origin.

The substance of the project and procedures associated with it have been fully explained to me, and all experimental procedures have been identified. I have had the opportunity to ask questions concerning any and all aspects of the project and any procedures involved.

Potential benefits from proposed treatment as well as possible risks and discomforts that may result from the taking of any medication or the performance of any procedure have been explained to me by Dr._____. I have been informed of possible alternatives (if treatment is involved) available as a course of treatment. I am aware that I may withdraw my consent at any time and that such withdrawal will not restrict access to health care services normally available at the University Hospitals. I acknowledge that no guarantee or assurance has been given by anyone as to results to be obtained.

I have been informed that in the event I suffer physical injury as a consequence of the investigation, I will receive appropriate medical treatment for that injury. I also understand I will receive no financial compensation from the University for any injuries resulting from the investigation.

Doctor:_____

_____
Signature of Subject

Witness:_____

If relative or legal representative signs, indicate relationship or other authority.

Date:_____

Time:_____ a.m. p.m.

Revised: 1/79

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7.

JOSEPH MANDEL BARON, M.D.

Curriculum Vitae

BIRTHDATE AND PLACE OF BIRTH: May 10, 1938 - Oak Park, Illinois

MARITAL STATUS: Married - 3 Children

EDUCATION:

Roosevelt High School, Chicago, Illinois	1950-54
College of University of Chicago	1954-58
University of Chicago - School of Medicine	1958-62
University of Chicago - B.S. (Biochemistry)-honors	1958
University of Chicago - M.D. - honors	1962
University of Chicago - M.S. (Pharmacology)	1962

POSITIONS:

NSF Summer Research Fellow	1960
Graduate Teaching Assistant, Department of Physiology (cardio-respiratory physiology) University of Chicago	1961
Intern (straight medicine) - University of Chicago	1962-63
Junior Assistant Resident (Medicine) - University of Chicago	1963-64
Research Associate, National Institutes of Health, Bethesda, Maryland (Laboratory of General and Comparative Biochemistry - National Institute of Mental Health). (military service)	1964-66
Chief Resident and Instructor - Department of Medicine - University of Chicago	1966-67
Instructor - Department of Medicine (Section of Hematology) - University of Chicago	1967-70
Assistant Professor - Department of Medicine (Section Of hematology) - University of Chicago	1970-74
Associate Professor - Department of Medicine (Section of Hematology/Oncology) - University of Chicago	1975-

SOCIETIES:

Phi Beta Kappa
Alpha Omega Alpha
Sigma Xi
American Society of Hematology
American Federation for Clinical Research
Chicago Society of Internal Medicine

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JOSEPH MANDEL BARON, M.D.

Curriculum Vitae

MEDICAL LICENSURE:

Illinois, California

RESEARCH INTERESTS:

Cellular physiology - differentiation and catabolic mechanisms

AWARDS:

Goethe Award - University of Chicago	1955
Student Aide - University of Chicago	1957
Borden Undergraduate Research Award in Medicine	1962

CERTIFICATION:

Diplomate, American Board of Internal Medicine	1969
Fellow, American College of Physicians	1972
Diplomate, Subspecialty of Hematology	1972
Diplomate, Subspecialty of Oncology	1975

JOSEPH MANDEL BARON, M.D.

Curriculum Vitae

BIBLIOGRAPHY:

Abstracts

1. Yachnin, S., Svenson, R.H., Allen, L.W., and Baron, J.M. The Biologic Properties of Mitogenic Proteins Derived from Phaseolus vulgaris. J. Clin. Invest. Vol. 48: p. 91a, June, 1969

2. Yachnin, S., Allen, L.W., Baron, J.M., and Svenson, R. Potentiation of Lymphocyte Transformation by Membrane-Membrane Interaction. Fourth Leukocyte Culture Conference, Hanover, N.H., June 26-28, 1969. Semiannual Reprot to the Atomic Energy Commission, March, 1970.

3. Henry Gerwurz, Joseph Baron, Oscar D. Ratnoff, and Stanley Yachnin. Coagulation-Associated Consumption of Complement: A Case with Dissociation between Serum and Plasma Complement Activities. J. Clinical Investigation, Vol. 49, No. 6., p. 33a, June, 1970.

4. Baron, J.M., Johnson, S., and Svenson, R.H. Decreased and Delayed Phytohemagglutinin (PHA) Binding by Chronic Lymphocytic Leukemia Lymphocytes. Clinical Research, Vol. 19, No. 2, p.411, April, 1971.

5. H. Gewurz, B. McLeod, J. Baron, and S. Yachnin. Nonimmune Activation of Complement: Two New Phenomena. Clinical Research, November, 1972.

6. F.L. Fishman, J.M. Baron, and A. Orlina. Non-Oxidative Hemolysis Due to Salicylazosulfapyridine: Evidence for an Immune Mechanism, Gastroenterology. Gastroenterology, 64(4):727, 1973 (April).

7. Lester, E.P., Miller, J.B., Baron, J.M., and Yachnin, S. Inhibition of Human Lymphocyte Transformation by Human Alpha Fetoprotein (HAFP); Studies on the Mode of HAFP Action and the Role of HAFP Polymorphism. Clin. Res. 25:45a, 1977.