

AD-A057 055

CALIFORNIA UNIV SAN FRANCISCO

F/G 6/3

THE MECHANISM OF BLOOD FUNCTION AND PRODUCTION AFTER INJURY. (U)

JUL 78 G F SHELDON

DADA17-72-C-2030

NL

UNCLASSIFIED

1 OF 1  
ADA  
057055

17  
17



END

DATE

FILMED

9 78

DDC

72

DDC FILE COPY AD A 0 5 7 0 5 5

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM	
1. REPORT NUMBER <b>6</b> See A038769	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER <b>119</b>	
4. TITLE (and Subtitle) The Mechanism of Blood Function and Production After Injury.		5. TYPE OF REPORT & PERIOD COVERED Annual Progress Report. 1977- <del>1978</del> 1978	
6. AUTHOR(s) <b>10</b> George F./Sheldon, MD		7. PERFORMING ORG. REPORT NUMBER	
<b>LEVEL</b>		8. CONTRACT OR GRANT NUMBER(s) DADA 17-72-C2030	
		9. PERFORMING ORGANIZATION NAME AND ADDRESS University of California San Francisco, CA 94143	
10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS		11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command, Washington, DC	
12. REPORT DATE <b>11</b> 25 Jul 1978		13. NUMBER OF PAGES 13	
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) <b>12</b> 18p.		15. SECURITY CLASS. (of this report)	
15a. DECLASSIFICATION/DOWNGRADING SCHEDULE		16. DISTRIBUTION STATEMENT (of this Report) Approved for public release; distribution unlimited	
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		<div style="border: 2px solid black; padding: 5px; text-align: center;"> <p>DDC RECEIVED AUG 3 1978 RESOLVED E</p> </div>	
18. SUPPLEMENTARY NOTES THIS DOCUMENT IS BEST QUALITY PRACTICABLE. THE COPY FURNISHED TO DDC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.			
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Oxyhemoglobin dissociation curve, P <sub>50</sub> , erythropoietin, and parenteral nutrition.			
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The studies examine the cause of the aregenerative anemia caused by malnutrition. Rats on a 2% protein depletion diet rapidly became anemic and had low erythropoietin values. Refeeding by oral and total parenteral nutrition restored erythropoietin values to normal. These studies confirm the role of erythropoietin in the anemia of malnutrition. They also establish that intravenous, as well as oral provision of nutrients return erythropoietin values to normal.			

387 756 78 07 31 247 AB



THE MECHANISM OF BLOOD FUNCTION AND PRODUCTION AFTER INJURY

ANNUAL PROGRESS REPORT

George F Sheldon, MD

July 25, 1978

Supported by

US ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND  
Washington, DC 20314

Contract No DADA 17-72-C2030 ✓

University of California  
San Francisco, California 94143 ✓

Approved for public release; distribution unlimited

78 07 31 247

Contract #DADA 17-72-C2030

Introduction

Protein calorie malnutrition (PCM) is universally accompanied by anemia. Paradoxically patients who receive total parenteral nutrition (TPN) frequently become anemic in spite of seemingly adequate quantities of calories, protein, vitamins and micronutrients. The anemia associated with both malnutrition and hyperalimentation is usually normochromic microcytic and is unaccompanied by reticulocytosis, in the presence of adequate iron stores (5). These chronic aregenerative anemias could be due to inadequate erythropoietin synthesis, deficiency of micro or macro-nutrients, or failure of the bone marrow to respond to erythropoietin stimulus.

Reissmann in 1964 performed a series of experiments designed to assess the effect of a protein depletion diet on erythropoietin synthesis in rats (7,8). His experiments demonstrated that erythropoietin synthesis was depressed by protein depletion, but exogenous administration of erythropoietin stimulated production of red cell precursors by the bone marrow, suggesting that the anemia of protein depletion was due to inadequate erythropoietin synthesis (3). When the protein depleted rats were fed a normal diet, erythropoietin synthesis returned to normal with eventual normalization of red cell mass.

The purpose of this experiment was to determine if total parenteral nutrition (TPN), like oral alimentation, could restore erythropoietin synthesis in a protein depleted rat with predictably deficient erythropoietin synthesis. As normally fed rats received TPN as controls, it was also possible to compare the effect of intravenous and oral nutrition in rats with erythropoietin synthesis unaltered by a protein deficient diet.

Material and Methods

The experimental design (Table 1) consisted of a 3-day period of (Phase I) dietary conditioning followed by a 7-day treatment period

(Phase II), which concluded by measuring erythropoietin stimulating factors (ESF). Male Sprague-Dawley rats weighing approximately 142 grams were initially divided into 2 groups, for dietary conditioning. The control group of 33 rats were fed ad lib Berkeley Standard mouse and rat diet for 30 days.\* The protein depletion group of 36 rats was fed a 2% agar diet ad lib for 30 days, also.\*\* After the 30-day period of dietary conditioning, both normally fed and protein depleted animals were divided into 3 nutritional sub-groups for a 7-day period; at the end of Phase II erythropoietin stimulating factor (ESF) was measured (Table I).

The 3 sub-groups of both the normal fed and protein depleted rats were comparable: Group A received total parenteral nutrition, Group B was fed a 2% agar protein depletion diet, and Group C received the standard laboratory diet.

Total parenteral nutrition in the rats was performed by placement of a silastic catheter through the jugular vein into the right atrium, after anesthesia was induced by methoxyflourane (10). Catheter fixation to the rat's neck muscles was performed using Cox's method (1). Because of the catheter length and use of a swivel, the animal had unrestrained movement in a metabolic cage. The total parenteral nutrition solution employed consisted of 4.25% Fre-Amine II in 30% dextrose with added vitamins and micronutrients at 0.4 ml/gm/qd (Table III).

Erythropoietin stimulating factor (ESF) was determined by the bioassay method of Fogh (2). The Sprague-Dawley rats were placed in a specially designed chamber which allowed continuation of their nutritional program during the period of hypoxia required to maximally stimulate ESF production. Using a flow-through system, the rats were subjected to a 5-hour period of hypoxic environment consisting of 8.8% oxygen, 90% nitrogen,

\* Feedstuffs Processing Co., San Francisco, CA.

\*\* U.S.P. XV, ICN Pharmaceuticals, Cleveland, Ohio

and <1% carbon dioxide. Following the period of hypoxic exposure, rats were anesthetized with methoxyflurane and exsanguinated by aortic puncture. The rat blood was collected without anticoagulant and the serum was eluted from the clot and frozen.

Erythropoietin stimulating factor was determined by Fogh's hypoxic mouse bio-assay (2). The method uses a mouse made polycythemic by several weeks of exposure to carbon monoxide. When a hematocrit of 70% or more is obtained, iron is administered to the animal. Following this preparation, serum from the Sprague-Dawley rat was injected into the plethoric mouse. Erythropoietin stimulating factor (ESF) was determined by the percent of radioactive iron ( $^{59}\text{Fe}$ ) uptake induced by the administered rat serum in the polycythemic mouse.

### Results

During the 30-day period of dietary conditioning, rats fed a normal diet (Group I) gained  $106.4 \pm 22.5$  grams. The animals fed a 2% agar protein depletion diet (Group II) lost  $37.1 \pm 6.6$  grams during the same 30-day period. No rats died during the period of nutritional conditioning. (Fig. I) (Table III)

During the 7-day treatment period, 6 rats receiving TPN died of air embolism and are not included in the results. Rats (I-C) who were fed the regular diet for the 30-day conditioning and the 7-day study period are the normals for the experiment and had ESF values of  $13.54 \pm 5.33\%$   $^{59}\text{Fe}$  uptake. The protein depleted rats had significantly lower ESF values at both 7 days ( $6.41 \pm 3.33$ ,  $p < 0.001$ ) and 30 days ( $4.88 \pm 1.88$ ,  $p < 0.001$ ) than the normally fed rats. (Fig. II) (Table IV)

When the rats which were subjected to 30 days of protein (Fig. III) depletion were returned to a normal oral diet, ESF values were significantly higher ( $16.58 \pm 3.81$ ) than ESF values during protein depletion. ( $p < 0.001$ ). They were also higher than the normal fed animals ( $p < 0.05$ ).

The highest ESF values occurred in the 2 groups of rats which received nutritional support by total parenteral nutrition (I-A,II-A). The rats administered TPN after 30 days of eating a 2% agar protein depletion diet had ESF values of  $(17.32 \pm 2.79)$  which were significantly higher than normal ( $p < 0.001$ ) and comparable to the group of rats whose protein repletion was by oral feeding. Of particular interest was the high ESF values  $(17.66 \pm 4.33)$  found in intravenously nourished rats who were fed a regular diet prior to receiving TPN (I-A). The ESF values in the normally fed rats who received TPN was significantly higher than normal ( $p < .001$ ), and significantly higher than the ESF values of the orally repleted protein deficient rat (II-C), ( $p < .001$ ). The ESF values in the normally fed and protein depleted groups which were administered TPN (I-A, II-A) were not significantly different.

#### Discussion

These studies are in agreement with Reissmann's experiments which revealed that 30 days of a diet of 2% agar protein depletion resulted in decreased synthesis of erythropoietin in rats. In addition, our data indicates that ESF synthesis decreases soon after the initiation of a protein depletion diet (7 days), suggesting that levels of ESF sufficient to stimulate the bone marrow to produce red cells become inadequate early in the development of calorie protein malnutrition. The lack of red cell production secondary to decreased erythropoietin could account for the anemia which accompanies calorie protein malnutrition.

After Reissmann found that exogenous administration of erythropoietin stimulated the bone marrow to produce reticulocytes in the protein depleted rats, Naets and Wittek found a absence of reticulocytosis in starved rats which were administered erythropoietin (6). Both Reissmann and Naets' experiments suggest that the totipotential bone marrow stem cell retains the capability of becoming a mature erythrocyte in the presence

of a severely protein restricted diet, a capability which is lost when protein is completely eliminated from the diet (9). The anemia of starvation then is probably due to developmental failure of bone marrow stem cells as well as decreased erythropoietin synthesis, unlike the anemia of protein depletion, which is due to decreased erythropoietin synthesis alone.

Nutritional repletion of protein depleted rats by oral refeeding predictably was associated with rapid recovery of erythropoietin synthesis. In the rats which were fed a normal diet after 30 days of a protein deficient diet, ESF values were significantly higher than normal, suggesting that erythropoietin may be a priority protein.

When total parenteral nutrition was the method of nutritional repletion employed, ESF values were significantly higher than normal and comparable to ESF values in orally repleted animals that had calorie protein malnutrition. Attainment of ESF values by the intravenous route which are comparable or higher than those obtained by oral refeeding, indicates that digestion by the gastrointestinal tract is not essential for nutrient material to be utilized in the synthesis of erythropoietin, a complex glycoprotein.

The normally fed rats when placed on TPN for 7 days had the highest ESF values of all groups. The high ESF values in normally fed rats on TPN are probably due to the continuous administration of nutritional substrate. It is possible that intravenous feeding produces a mass action effect on some aspects of protein synthesis, such as ESF. As these animals received TPN for only 7 days, it is not known if polycythemia would have resulted from prolonged elevated ESF values secondary to TPN.

The high ESF values found in both protein depleted and normal animals which were administered TPN suggests that the anemia frequently found in patients receiving TPN is probably not due to inadequate ESF

production. Alternative explanations for the TPN associated anemia would include accelerated red cell destruction, lack of a micro or macronutrient, or an unresponsive bone marrow (4).

#### Conclusion

Rats fed a protein restricted diet have a diminished capability to synthesize erythropoietin. When a normal diet is given to protein depleted rats, ESF values become higher than normal within 1 week of refeeding.

When TPN is administered to rats, regardless of the animal's nutritional state, marked elevation in ESF occurs. The data suggests that TPN, like oral feeding, will support ESF synthesis. Moreover, rats receiving continuous intravenous feeding of dextrose and amino acids appear to have accelerated synthesis of erythropoietin.

The anemia which frequently accompanies TPN is probably not secondary to inadequate formation of erythropoietin.

## References

1. Cox C.E., Beazley R.M.: Chronic venous catheterization: a technique for implanting and maintaining venous catheters in rats. *J of Surg Res* 18:607-610, 1975.
2. Fogh J.: A sensitive erythropoietin assay on mice exposed to CO hypoxia. *Scandinav J Clin Lab Invest* 18:33-44, 1966.
3. Ito K. and Reissmann K.R.: Quantitative and qualitative aspects of steady state erythropoiesis induced in protein-starved rats by long-term erythropoietin injection. *Blood* 27:343, 1966.
4. Krantz S.B.: Recent contributions to the mechanism of action and clinical relevance of erythropoietin. *J of Lab and Clin Med* 82: 847, 1973.
5. Kurnick J.E., Ward H.P. and Pickett J.C.: Mechanism of the anemia of chronic disorder. *Arch Intern Med* 130:323-326, 1972.
6. Naets J.P. and Wittek M.: Effect of starvation on the response to erythropoietin in the rat. *Acta Haemat* 52:141, 1974.
7. Reissmann K.R.: Protein metabolism and erythropoiesis: I. the anemia of protein deprivation. *Blood* 23:127, 1964.
8. Reissmann K.R.: Protein metabolism and erythropoiesis: II. erythropoietin formation and erythroid responsiveness in protein-deprived rats. *Blood* 23:146, 1964.
9. Reissmann K.R. and Samorapoompickit S.: Effect of erythropoietin on proliferation of erythroid stem cells in the absence of transplantable colony-forming units. *Blood* 36:287, 1970.
10. Steiger E., Vars H.M. and Dudrick S.J.: A technique for long-term intravenous feeding in unrestrained rats. *Arch Surg* 104: 330-332, 1972.

TABLE I: EXPERIMENTAL GROUPS

<u>group</u>	<u>Phase I</u> (30 days)	<u>Phase II</u> (7 days)
I.	Standard Laboratory Diet	A. Total Parenteral Nutrition
	Standard Laboratory Diet	B. Protein Depleted Diet
	Standard Laboratory Diet	C. Standard Laboratory Diet
II.	Protein Depletion Diet	A. Total Parenteral Nutrition
	Protein Depletion Diet	B. Protein Depletion Diet
	Protein Depletion Diet	C. Standard Laboratory Diet

TABLE II: TPN SOLUTION

30% Dextrose 4.25% Amino Acid

Electrolytes: (meg per liter)

NaCl 20

K<sub>2</sub>HPO<sub>4</sub> 15CaCl<sub>2</sub> 9MgSO<sub>4</sub> 8Vitamins: (ml per liter)

M.V.I. 5

TABLE III: PHASE I WEIGHT CHANGES

	<u>gms</u>	<u>n</u>
Standard Laboratory Diet	106.4 $\pm$ 22.5 gain	33
Protein Depletion Diet	37.1 $\pm$ 6.6 loss	36

TABLE IV: PHASE II WEIGHT CHANGES

<u>group</u>	<u>gms</u>	<u>n</u>	<u>group</u>	<u>gms</u>	<u>n</u>
	A. 38.7 $\pm$ 8.2 gain	9	I	A. 41.0 $\pm$ 7.9 gain	9
I	B. 14.7 $\pm$ 5.8 loss	15	II	B. 6.1 $\pm$ 4.0 loss	11
	C. 13.7 $\pm$ 11.5 gain	6		C. 51.8 $\pm$ 10.9 gain	12

### Legend for Illustrations

FIGURE I. During Phase I, the 30-day period of dietary conditioning, the rats fed a standard diet increased their weight by approximately 25%; rats fed a 2% agar protein depletion diet were approximately 25% lighter after 30 days.

FIGURE II. Weight changes during Phase II, the 7-day period of refeeding, which followed the 30-day (Phase I) period of dietary conditioning.

FIGURE III. ESF values in the 6 groups of rats following Phase I (dietary conditioning) and Phase II (7 days of refeeding).

The highest ESF values are found in rats who received TPN regardless of their prior state of dietary conditioning (standard diet or 2% agar protein depletion).

Rats receiving a standard diet demonstrated a pronounced fall in ESF values after only 7 days of protein depletion diet and rats continued on the depletion diet had still lower ESF values.

Rats fed the standard diet during Phase I and Phase II (37 days) are the normal ESF values ( $13.41 \pm 5.33\%$   $^{59}\text{Fe}$ ). Rats who received 2% agar protein depletion diet during the 30 days of Phase I, who then received a standard diet during the 7 days of Phase II, had ESF values which were significantly higher than normal and comparable to both protein depleted and normally fed animals which received TPN.

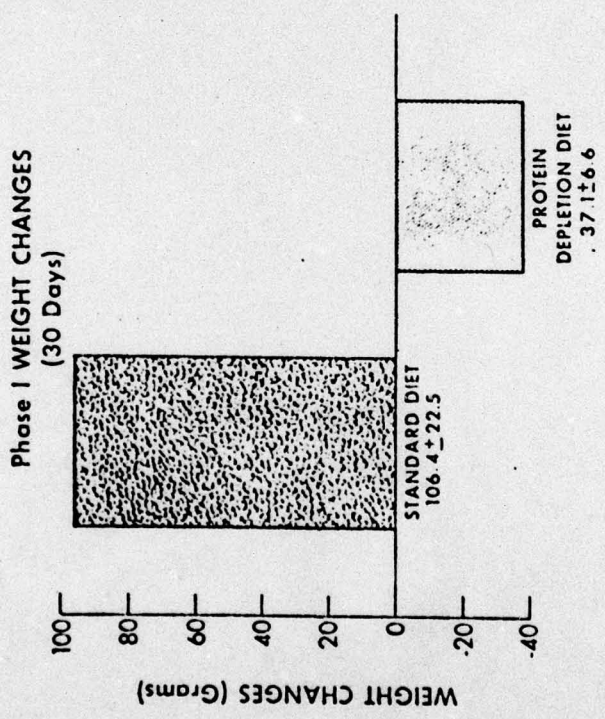


FIGURE I

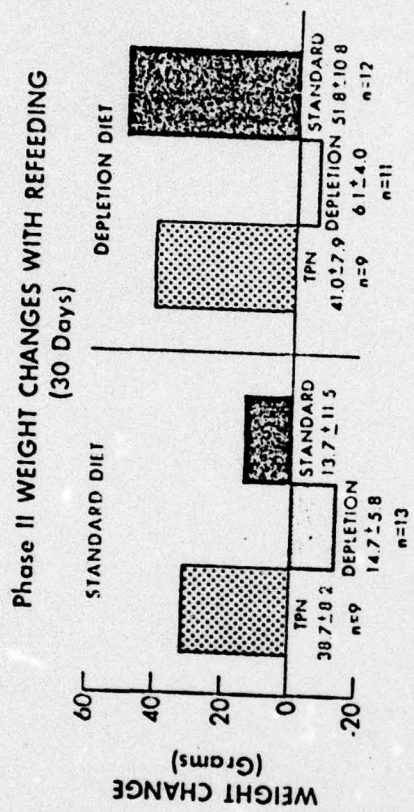


FIGURE II

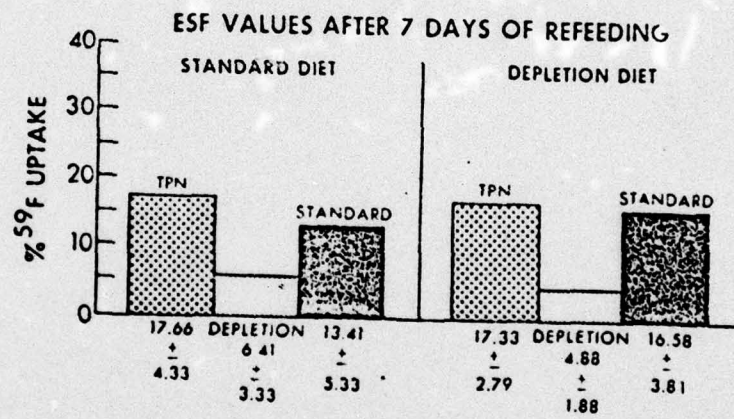


FIGURE III

PUBLICATIONS CREDITED TO ARMY CONTRACT

1. Plzak, L.F., Watkins, G., Sheldon, G.F.: Hyperalimintation and the oxy-hemoglobin dissociation curve. Chapter in Intravenous Hyperalimintation, pp. 196-203, Lea and Febiger, publishers, 1972.
2. Sheldon, G.F.: Hyperphosphatemia, hypophosphatemia in the oxy-hemoglobin dissociation curve. Journal of Surgical Research, 14:367-372, 1973.
3. Sheldon, G.F.: Defective hemoglobin function: a complication of hyperalimintation. Journal of Trauma, 13:971-979, 1973.
4. Grzyb, S., Jelinek, C., Sheldon, G.F.: Phosphate depletion syndrome: Relation to caloric intake and phosphate infusion. Surgical Forum, 24:103-104, 1973.
5. Sheldon, G.F., Holcroft, J., Heppenstall, R.B., Fuchs, R., Hunt, T.K.: Massive transfusion: A metabolic and hemodynamic lesion. Surgical Forum, 24:17-18, 1973.
6. Mentzer, W.C., Addiego, J., Sheldon, G.F., Goldman, P., Kan, Y.W.: Modulation of oxygen affinity by phosphate in sickle cell anemia. Clinical Research, 22:225a, 1974.
7. Heppenstall, R.B., Littooy, F., Fuchs, R., Sheldon, G.F., Hunt, T.K.: Gas tension in healing tissue of traumatized patients. Surgery, 75:874-880, 1974.
8. Sheldon, G.F., Jelinek, C., Fuchs, R.: The role of inorganic phosphate in recovery from transfusion. Surgical Forum, 25:430, 1974.
9. Sheldon, G.F.: Transfusion practices in trauma patients. California Medicine, July 1975.
10. Sheldon, G.F., Lim, R.C., Blaisdell, F.W.: The use of fresh blood in critically injured patients. Journal of Trauma, August 1975.
11. Fuchs, R., Holcroft, J.: The HP 9810A at San Francisco General Hospital. Hewlett Packard Keyboard, 6:4, 1974.
12. Mentzer, W.C., Addiego, J., Sheldon, G.F., Goldman, P., Kan, Y.W.: Modulation of oxygen affinity by phosphate in sickle disease. Abstracts of the First International Symposium on Sickle Disease. 345-364, 1974.
13. Sheldon, G.F., Grzyb, S.: Phosphate depletion and repletion: Relation to parenteral nutrition and oxygen transport. Annals of Surgery, 182:683-689, 1975.
14. Littooy, F., Fuchs, R., Hunt, T.K., Sheldon, G.F.: Tissue oxygen as a real-time measure of oxygen transport. J Surg Res 20: 321-325, 1976.

15. Sheldon GF and Fuchs R: In vivo comparison of blood preservatives J. of Trauma 17:505-509, 1977.
16. Sanders R, Garcia J, Sheldon G, Schooley J and Fuchs R: Erythropoietin elevation in anemia of thermal injury. Surgical Forum 27: 71-2, 1976.
17. Sanders R, Garcia J, Sheldon GF, Schooley J and Fuchs R: Erythropoietin synthesis during total parenteral nutrition. J of Surg Res 22:649-653, 1977.
18. Fresh whole blood: Less than the sum of its parts. EMERGENCY MEDICINE, GF Sheldon (consulting editor) 8:100-107.
19. Sheldon GF and Fuchs R: Arteriography: Another low phosphate syndrome? J of Surg Res 22:679-682, 1977.
20. Sheldon GF: The Role of 2,3 Diphosoglycerate in Oxygen Transportation. Vox Sanguinis, 1977.
21. Sheldon GF: Blood and Blood Products, 6th edition Handbook of Surgery, Chapter II, Postoperative and Preoperative Care, in General Surgery, T Schrock, editor, R Jones, Co, 1978.
22. Sheldon GF, Fuchs R and Carpenter G: The Storage Lesion of Blood: Role in Massive Transfusion and Microaggregate formation. Chapter in Microaggregates: Experimental and Clinical Aspects, Warren H Green, Inc. (to be published 1978)
23. Sheldon GF and Fuchs R: In vivo comparisons of blood preservatives. Rev of Surg, 1977. (abstract)
24. Sheldon GF, Sanders R, Fuchs R, Garcia J and Schooley J: Metabolism oxygen transport and erythropoietin synthesis in the anemia of thermal injury. Amer J of Surg 135:406-411, 1978.
25. Sheldon GF and Petersen S: Effect of protein:calorie malnutrition on cardiac and pulmonary function and oxygen transport. Accepted for publication Amer Med Assn, San Antonio, TX, 1977.

ACCESSION for	
NTIS	White Section <input checked="" type="checkbox"/>
DDC	Buff Section <input type="checkbox"/>
UNANNOUNCED	<input type="checkbox"/>
JUSTIFICATION	
BY	
DISTRIBUTION/AVAILABILITY CODES	
Dist.	AVAIL. SPECIAL
A-23 14	