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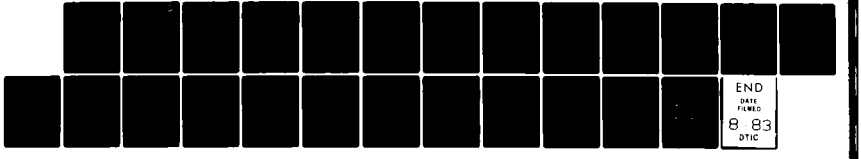

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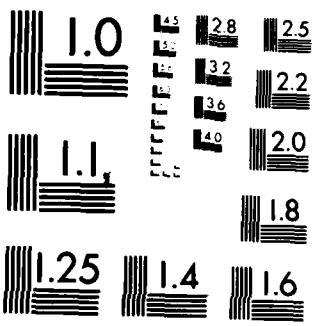

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THE MECHANISM OF BLOOD FUNCTION AND PRODUCTION AFTER INJURY

ANNUAL PROGRESS REPORT

GEORGE F. SHELDON, M.D.

FEBRUARY 1980

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University of California
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Protein depletion in rats is associated with hemoconcentrated diminution of red cell mass and loss of erythropoiesis. Restoration of nutrition by diet or total parenteral nutrition (TPN) is accompanied by weight gain and restoration of red cell mass. Intravenous repletion of both normal and protein depleted rats, unlike orally refed cohorts, was associated with elevated 2,3 diphosphoglycerate (2,3 DPG) values and anemia. It is postulated that elevated red blood cell 2,3 DPG values in the intravenously refed rats interacted with hemoglobin to (over)

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20. (Continuation): enhance oxygen unloading, thereby lowering the hypoxic stimulus for erythropoietin production, and ablating the hypoxic stimulus to the kidney. Because of the lack of a hypoxic stimulus to the kidney, an aregenerative anemia persisted.

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SUMMARY

Protein depletion in rats is associated with hemoconcentrated diminution of red cell mass and loss of erythropoiesis. Restoration of nutrition by diet or total parenteral nutrition (TPN) is accompanied by weight gain and restoration of red cell mass. Intravenous repletion of both normal and protein depleted rats, unlike orally refed cohorts, was associated with elevated 2,3 diphosphoglycerate (2,3 DPG) values and anemia. It is postulated that elevated red blood cell 2,3 DPG values in the intravenously refed rats interacted with hemoglobin to enhance oxygen unloading, thereby lowering the hypoxic stimulus for erythropoietin production, and ablating the hypoxic stimulus to the kidney. Because of the lack of a hypoxic stimulus to the kidney, an aregenerative anemia persisted.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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ARMY CONTRACT ANNUAL PROGRESS REPORT

Contract #DADA 17-72-C2030

THE MECHANISM OF BLOOD FUNCTION AND PRODUCTION AFTER INJURY

Introduction

Protein-calorie malnutrition is associated with an aregenerative anemia[1], and nutritional depletion is often a consequence of severe illness and injury. Intravenous hyperalimentation has been used extensively to provide nutritional support during the catabolic period associated with major surgical stress and trauma. Despite restitution of positive nitrogen balance and weight gain while receiving hyperalimentation, these critically ill patients often become anemic and require multiple transfusions.

The anemia associated with total parenteral nutrition is essentially the anemia which has been described in many chronic disease states[19]. It is an aregenerative, normochromic normocytic anemia, with low serum iron levels and depressed iron binding capacity. Bone marrow studies in these patients reveal hypoplastic erythroid elements and adequate iron stores. The etiology of this anemia is controversial. It may be related to shortened red cell survival, failure of the bone marrow to respond to erythropoietin or a deficiency in micro or macronutrients. The anemia is probably not secondary to an inability to synthesize erythropoietin[12]. Moderate anemia (20-50% reduction in red cell mass) affects oxygen transport, not by raising cardiac output, but by increasing oxygen extraction[18]. Oxygen transport in anemia is further compensated by elevation of red cell 2,3 diphosphoglycerate (2,3 DPG). Diphosphoglycerate binds to hemoglobin within the red cell and in association with a number of biochemical and environmental factors lowers hemoglobin affinity for oxygen, thus increasing oxygen delivery to the tissues[13].

Correction of the anemia associated with hyperalimentation, or nutritional depletion can be managed with intermittent blood transfusions, however, recovery

from anemia depends on stimulation of erythropoiesis. Moreover, red cell production is stimulated by the hormone erythropoietin, a glycoprotein which is synthesized primarily in the kidney[6]. The major factor responsible for initiating erythropoietin synthesis appears to be relative tissue hypoxia in the renal cortex. Elevated levels of 2,3 DPG, often seen in association with chronic anemias,[3,9] lower the affinity of hemoglobin for oxygen, and improve oxygen delivery to the tissues. Because of its ability to increase oxygen unloading, 2,3 DPG may play a role in eliminating the hypoxic stimulus for erythropoiesis[11,16]. This study was designed to determine if total parenteral nutrition could restore the red cell mass deficit incurred with protein depletion and to assess the possible regulatory role of 2,3 DPG on erythropoiesis during hyperalimentation.

MATERIALS AND METHODS:

The experimental design consisted of a 14 day dietary conditioning period and a 21 day treatment period. Male Sprague-Dawley rats weighing approximately 140 grams were divided into two groups for dietary conditioning. Twenty rats received standard laboratory rat food (Simonsen Laboratories, 22% protein) ad lib for 14 days and 19 cohorts were given 2% agar protein depletion diet (Microbiological Supply) ad lib, also for 2 weeks. Following the period of dietary conditioning, five rats from each group were sacrificed to serve as controls. The remaining rats in each group were refed for 21 days either orally with a standard diet (Standard) or intravenously (TPN) with a dextrose-amino acid solution. The parenteral nutrition was administered in an unrestrained rat model which has previously been described[12]. The solution used for total parenteral nutrition consisted of crystalline amino acids (3.5% Aminosyn, Abbott

Laboratories) and 25% glucose. Additional amino acids were added to more closely approximate the basal requirements of the rat (Table I).[2] Choline which has been shown to be an essential nutrient for the rat was also added. Trace metals (copper, cobalt, manganese, iodine and zinc) as well as multiple vitamins were also administered. Biweekly supplementation with iron, cyanocobalamin, vitamin K, and folic acid was administered by intramuscular injection (Table II). Intravenous fat emulsions were not provided. The rate of infusion was 0.4 ml/gram body weight per day.

Hematocrits, hemoglobin values and reticulocyte counts were measured by standard laboratory techniques. Red cell mass determinations were obtained using the radioactive ^{59}Fe labeled red cell dilution method described by Garcia[7]. Red cell 2,3 diphosphoglycerate levels were measured using a commercial kit (Sigma) for enzymatic analysis according to the method of Keitt[8]. Statistical significance was determined by the Kruskal-Wallis test for nonparametric multiple comparisons and by the Newman-Kuels test for parametric data.

RESULTS:

During dietary conditioning, rats receiving the protein depletion diet lost 47.9 $\pm$ 4.0 grams (Table III). Weight gain in rats fed the standard diet during the 2 week dietary conditioning period was 98.8 $\pm$ 21.1 grams. Weight gain with parenteral refeeding was consistently less than rats repleted with standard laboratory diets (Figure 1). Previous experiments have shown this difference to be directly related to nitrogen intake (r^2=.92) which is higher in rats fed standard diets compared to rats nourished parenterally (unpublished data).

Control rats in the depleted group had significantly higher hematocrits than their standard cohorts ($p<.001$)(Figure 2). These values were also higher

than either parenterally nourished or orally repleted rats in the depletion group ($p<.001$). Rats maintained on standard diets had comparable hematocrit values to depleted controls, however they were significantly higher than cohort rats refed with total parenteral nutrition ($p<.001$).

Depleted controls all had reticulocyte counts below 0.1% showing suppression red cell production in response to protein depletion. Reticulocyte counts were elevated ($p<.001$) in standard and depleted rats receiving intravenous feeding as compared to controls indicating stimulation of erythropoiesis (Table IV) during parenteral nutrition.

Erythrocyte mass was significantly lower in depleted controls as compared to standard controls ($p<.001$) (Table IV). The standard and TPN repleted rats in each dietary group had higher red cell masses than controls indicating that parenteral nutrition as well as oral feeding can promote erythropoiesis ($p<.025$). Rats refed on standard diets, however, had higher red cell masses than rats repleted with TPN ($p<.001$) implying a "relative anemia" in parenterally nourished rats when compared to their oral refed cohorts. Intravenous repletion of normal and protein depleted rats was also associated with elevated 2,3 diphosphoglycerate levels ($p<.05$)(Table IV) suggesting partial compensation for anemic hypoxia by 2,3 DPG during total parenteral nutrition. When 2,3 DPG values were plotted against hemoglobin concentration in protein depleted and standard groups there was a significant negative correlation ($r=-.62$; $p<.001$)(Figure 3).

DISCUSSION:

Red cell production and function are dependent in part on protein synthesis. Protein depletion may occur through dietary deprivation or secondary to the catabolism associated with major illness or injury. The etiology of the anemia [illegible] results from protein deficiency is multifactorial and may be due to

a lack of micronutrients or inadequate protein synthesis to produce erythropoietin[10].

Our studies have demonstrated that removal of adequate protein from a rat's diet leads to a marked reduction in erythropoiesis, hemoconcentration and diminution of red cell mass, findings which are in agreement with the results of others[1]. Hematocrit values were found to poorly correlate with the red cell mass diminution that occurs with protein depletion and probably reflects the marked changes in body water distribution that occur with protein depletion and repletion. Recovery from protein depletion was associated with a reticulocyte response to dietary treatment and an early fall in hemoglobin concentration secondary to a rapid rise in a previously reduced plasma volume outstripping the regeneration of red blood cells.

Total parenteral nutrition like oral refeeding can promote erythropoiesis and restore red cell mass as it can rebuild body weight. However, oral repletion with standard diets tends to regenerate red cell mass and body weight faster than parenteral feedings. Previous experiments have shown this difference in weight gain to be primarily related to an increased nitrogen intake in rats fed standard diets when compared to rats given parenteral nutrition. (unpublished data)

This study demonstrates a "relative anemia" in rats repleted with total parenteral nutrition as compared to rats refed with standard diets. Sanders, and others,[12] have shown that the anemia associated with hyperalimentation is probably not due to an inability to synthesize erythropoietin and, infact, rats nourished intravenously have higher levels of erythropoietin in response to a hypoxic stimulus than do rats fed orally. Our data demonstrating elevated 2,3 DPG values in normal and protein depleted rats receiving intravenous hyperali-

mentation suggests that red cell glycolytic intermediates, primarily 2,3 DPG and ATP, enhance oxygen unloading, lower the hypoxic stimulus for erythropoietin production, and partially compensate for the "relative anemia" associated with total parenteral nutrition. Presumably, the increase in 2,3 DPG alters hemoglobin affinity for oxygen, increases the amount of tissue oxygen available to the renal regulatory center, and eliminates the stimulus for erythropoietin synthesis. This lack of erythropoietin results in failure of the bone marrow to produce adequate reticulocytes and a relative anemia persists.

There are several reports that suggest that 2,3 DPG modulates erythropoiesis by regulating hemoglobin-oxygen affinity. Pollock, and others,[11] showed that erythropoiesis in subhuman primates is retarded by augmented red cell glycolysis. They demonstrated that chronic infusion of dextrose and inosine phosphate resulted in elevation of red cell 2,3 DPG values and a shift of the oxyhemoglobin dissociation curve to the right. They postulated the change in hemoglobin-oxygen affinity led to the development of a progressive anemia observed in their study. The "physiologic anemia" of childhood is associated with elevated serum inorganic phosphate, 2,3 DPG values and P_{50} implying that enhanced red cell glycolysis lowers the hypoxic stimulus for erythropoietin production[4]. Stockman, and others,[15] found that premature infants with low hemoglobin values have higher 2,3 DPG and P_{50} values than infants with higher hemoglobin values. Because erythropoietin values were elevated in the infants with increased affinity of hemoglobin for oxygen, 2,3 DPG modulation of oxygen unloading capacity, not the hemoglobin concentration was considered to be the major regulating factor in erythropoietin synthesis and red cell production[15]. Elevated 2,3 DPG values have also been described with the anemia of thermal injury[14] and the anemia following trauma,[17] as well as a number of other chronic anemias,[3] suggesting that increased 2,3 DPG

concentration in the erythrocyte is a compensatory mechanism regulating tissue oxygen delivery and ultimately erythropoiesis.

The regulation of erythropoiesis is a complex entity involving a multitude of factors (Figure 4). The theory that erythropoietin synthesis and red cell production are modulated by alterations in hemoglobin-oxygen affinity due to enhanced red cell glycolysis is an oversimplification of an intricate biological feedback system. However, it seems evident from our data, and others[3, 4,5,14], that hemoglobin concentration is partially determined by red cell 2,3 DPG levels. Whether elevated 2,3 DPG levels are the cause or effect of low hemoglobin values observed during total parenteral nutrition needs further clarification.

SUMMARY:

Protein depletion in rats is associated with hemoconcentration, diminution of red cell mass, and suppression of erythropoiesis. Repletion with standard diets or total parenteral nutrition was associated with gain in body weight and regeneration of red cell mass. Hematocrit values did not reflect the degree of red cell mass diminution associated with protein depletion. Intravenous repletion of normal and protein depleted rats, unlike oral refed cohorts was associated with elevated 2,3 DPG values and a "relative anemia". It is postulated that elevated red cell 2,3 DPG lowers the hypoxic stimulus for erythropoietin production by enhancing oxygen unloading to the tissues, thus partially compensating for the anemia associated with total parenteral nutrition.

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Figure Legends:

Figure 1 - Weight change during repletion with standard diets (Standard) or total parenteral nutrition (TPN) in protein depleted (Depletion) and normal (Standard) rats.

Figure 2 - Hematocrit values during repletion in rats receiving standard diets or TPN.

Figure 3 - Correlation between hemoglobin and red cell 2,3 Diphosphoglycerate in protein depleted and standard groups (n=39)($r=-0.62$; $p < .001$)

Depleted controls; Depleted-standard; Depleted-TPN;
Standard controls; Standard-standard; Standard-TPN

Figure 4 - Protein-calorie malnutrition is associated with deleterious effects on all components of oxygen transport. Oxygen consumption (V_{O2}), cardiac output (Q_T), venous O_2 saturation (S_{VO2}), and arterial oxygen content (C_{aO2}) decrease; also Hgb values decrease as ESF decreases. Although P_{50} and 2,3 DPG values are normal with a stable V_{O2}, they increase when V_{O2} increases with refeeding, ablating the hypoxic stimulus for ESF production with persistence of an aregenerative anemia.

TABLE I

RAT PARENTERAL SOLUTION

Additive	Amount/Liter
Glucose - 25%	250 grams
Aminosyn - 3.5% (Abbott Labs)	
Additional Amino Acids	
Isoleucine	1.57 grams
Tyrosine	0.32 grams
Cysteine	1.00 grams
Additional Nutrients	
Choline chloride	0.15 grams
Trace Elements	
Cobalt	14.0 ucg
Copper	55 ucg
Manganese	100 ucg
Zinc	200 ucg
Iodine	7.5 ucg
Electrolytes	
Sodium chloride	25 mEq
Potassium chloride	4 mEq
Potassium phosphate	10 mEq
Magnesium sulfate	16 mEq
Calcium gluceptate	2.7 mEq
Multivitamins - MVI	5.0 cc

TABLE II

VITAMIN AND IRON SUPPLEMENTATION

NUTRIENT	AMOUNT/BIWEEKLY
Folic acid - (Folvite)	0.1 mg
Vitamin K (Aquamephyton)	1.0 mg
Vitamin B_{12} (Cyanocobalamin)	10 mcg
Iron - Fe Dextran	5 mg

TABLE III

WEIGHT CHANGES DURING DIETARY CONDITIONING AND REPLETION

		CONDITIONING			REPLETION		
		STAND	DEP	DEP-STAND	DEP-TPN	STAND-STAND	STAND-TPN
	INITIAL WEIGHT	WEIGHT GAIN	WEIGHT LOSS	WEIGHT GAIN	WEIGHT GAIN	WEIGHT GAIN	WEIGHT GAIN
MEAN	141.6	98.8	47.9	169.1	92.8*	115.3	47.1**
± S.D.	21.9	21.1	4.1	11.7	8.4	23.9	12.2
N	39.0	20.0	19.0	5.0	9.0	6.0	9.0

*$p < 0.001$
**$p < 0.01$

TABLE IV

ERYTHROPOIESIS DURING REPLETION

GROUP	n	RETICULOCYTE † COUNT (%)	RED CELL MASS (ml)	2,3 DPG (um/Hgb)
DEPLETION				
Control	5	<.01	2.68 ± 0.12**	18.0 ± 2.0
Standard	5	1.3 ± 0.3	6.06 ± 0.39	22.6 ± 2.2
TPN	9	4.9 ± 1.4**	3.73 ± 0.60**	24.5 ± 1.2
STANDARD				
Control	5	1.8 ± 0.8	5.39 ± 0.69	27.3 ± 2.2
Standard	6	0.6 ± 0.2	7.80 ± 0.78	23.6 ± 1.0
TPN	9	10.8 ± 6.4**	6.62 ± 0.61**	26.3 ± 1.7*

†± S.D.; **P <.001; *P >.05

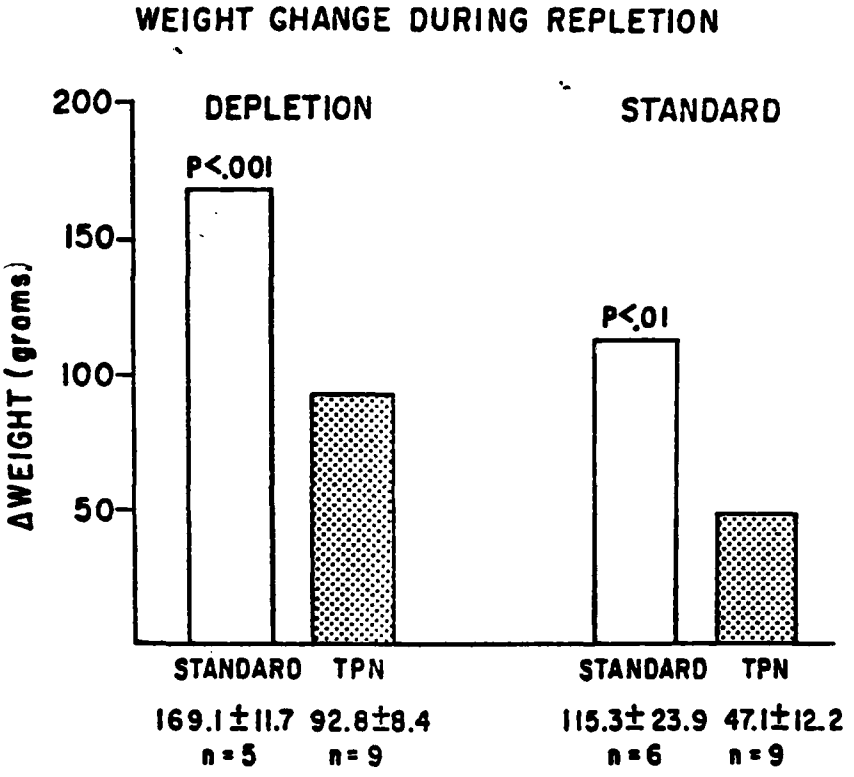
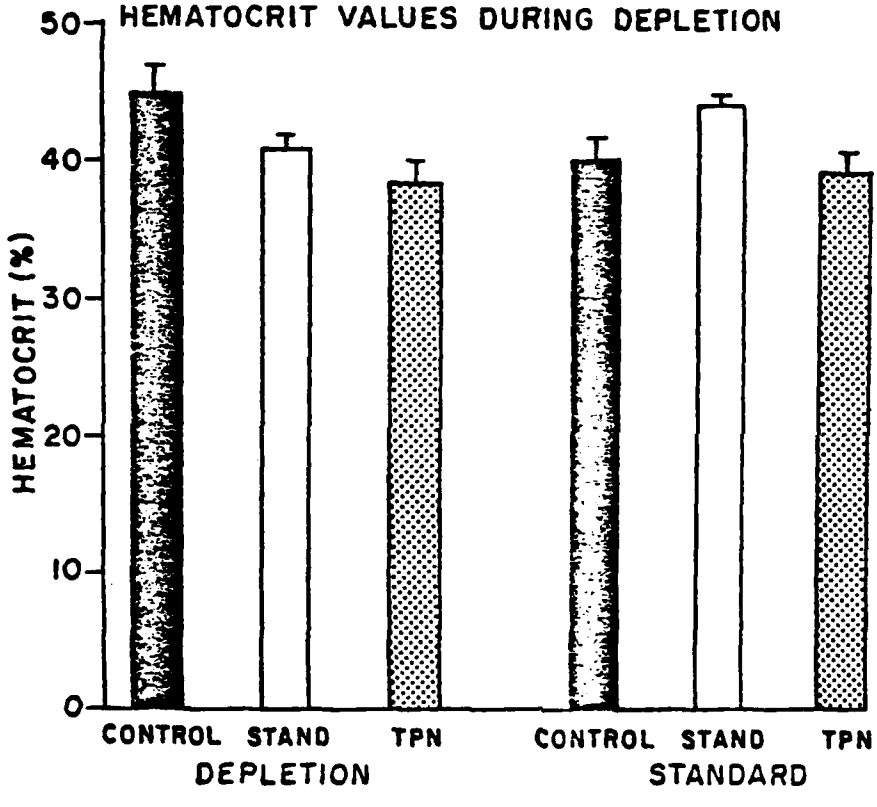

WEIGHT CHANGE DURING REPLETION
200
150
100
50
ΔWEIGHT (grams)
DEPLETION
STANDARD
P<.001
P<.01
STANDARD
TPN
STANDARD
TPN
169.1±11.7
n=5
92.8±8.4
n=9
115.3±23.9
n=6
47.1±12.2
n=9

Fig 1


HEMATOCRIT VALUES DURING DEPLETION
HEMATOCRIT (%)
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30
20
10
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CONTROL
STAND
TPN
CONTROL
STAND
TPN
DEPLETION
STANDARD

Fig 2

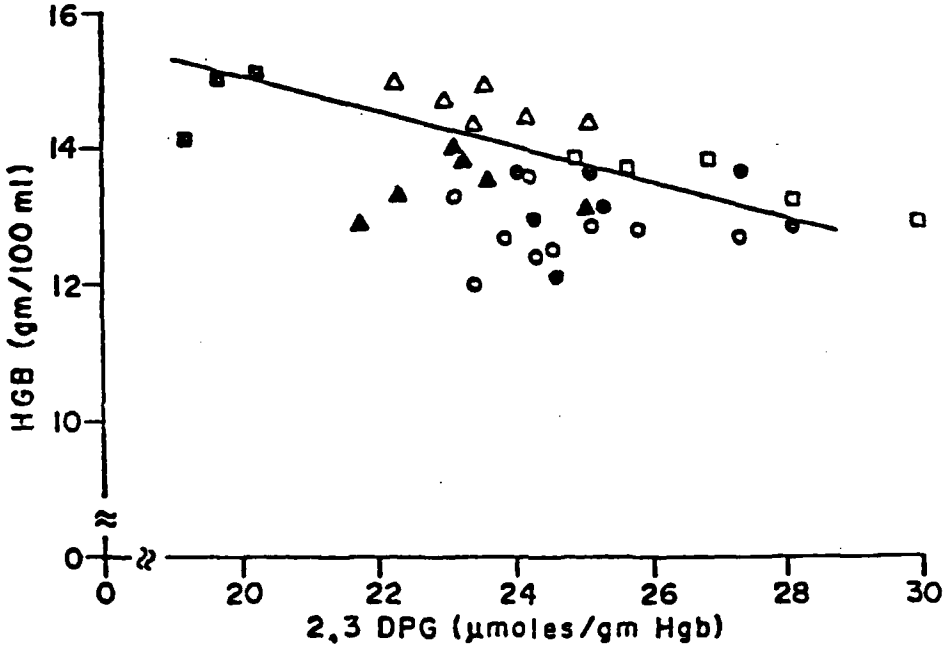

Fig 3

MALNUTRITION AND OXYGEN TRANSPORT

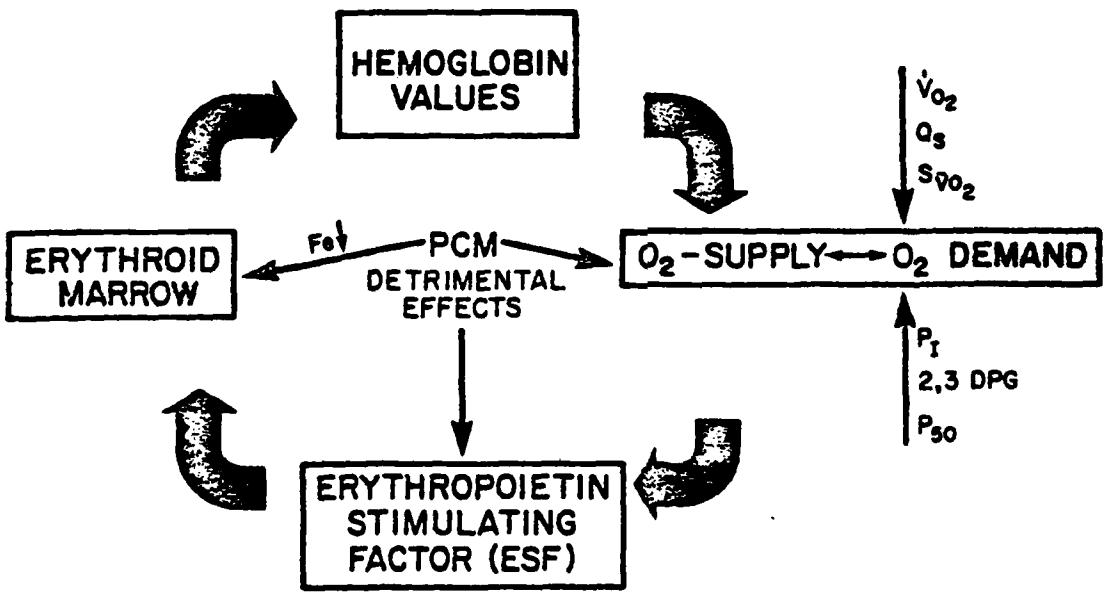

Fig 4


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