## The Mechanism of Blood Function and Production After Injury

### Abstract

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.

### Keywords

- Oxyhemoglobin dissociation curve, P50, erythropoietin, and parenteral nutrition.
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THE MECHANISM OF BLOOD FUNCTION AND PRODUCTION AFTER INJURY

ANNUAL PROGRESS REPORT

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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 (59Fe) 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 ± 22.5 grams. The animals fed a 2% agar protein depletion diet (Group II) lost 37.1 ± 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 ± 5.33%59Fe uptake. The protein depleted rats had significantly lower ESF values at both 7 days (6.41 ± 3.33, p<0.001) and 30 days (4.88 ± 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 ± 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


### TABLE I: EXPERIMENTAL GROUPS

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

### TABLE II: TPN SOLUTION

- **30% Dextrose 4.25% Amino Acid**
- **Electrolytes** (meg per liter):
  - NaCl: 20
  - K₂HPO₄: 15
  - CaCl₂: 9
  - MgSO₄: 8
- **Vitamins** (ml per liter):
  - M.V.I. 5
### TABLE III: PHASE I WEIGHT CHANGES

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<thead>
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<th>Diet</th>
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<td>Protein Depletion Diet</td>
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### TABLE IV: PHASE II WEIGHT CHANGES

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<th>Group</th>
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<td>38.7 ± 8.2</td>
<td>9</td>
<td>A</td>
<td>41.0 ± 7.9</td>
<td>9</td>
</tr>
<tr>
<td>I</td>
<td>14.7 ± 5.8</td>
<td>15</td>
<td>II</td>
<td>6.1 ± 4.0</td>
<td>11</td>
</tr>
<tr>
<td>C</td>
<td>13.7 ± 11.5</td>
<td>6</td>
<td>C</td>
<td>51.8 ± 10.9</td>
<td>12</td>
</tr>
</tbody>
</table>
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 ± 5.33% 59Fe). 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 1

Phase I WEIGHT CHANGES
(30 Days)

WEIGHT CHANGES (GRAMS)

- STANDARD DIET (-2.5)
- PROTEIN DELETION DIET 0.5/6
ESF VALUES AFTER 7 DAYS OF REFEEDING

STANDARD DIET

DEPLETION DIET

FIGURE III
PUBLICATIONS CREDITED TO ARMY CONTRACT


18. Fresh whole blood: Less than the sum of its parts. EMERGENCY MEDICINE, GF Sheldon (Consulting editor) 8:100-107.


