Protein Metabolism and Erythropoiesis. I. The Anemia of Protein Deprivation

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THE IMPORTANCE of dietary protein in red cell formation has been recognized largely through the classical work of Whipple and associates.1 The pertinent findings of these and subsequent studies can be summarized in three sets of observations: (1) An anemia develops in animals whose diet is deficient in protein,2-5 and markedly depressed reticulocyte counts6 and radioiron incorporations7 have revealed the non-regenerative nature of this anemia. In some species, a concomitant contraction of the plasma volume tends to mask the decline in red cell mass, and measurements of hemoglobin or corpuscular concentrations alone may be misleading.3 (2) An acceleration of erythropoiesis can be induced by subjecting a protein-deprived animal to bleeding.1-6 As the result, considerable amounts of hemoglobin are regenerated, although less than in a protein-supplemented animal. The necessary amino acids are drawn from the body\textsuperscript{\textregistered} protein pool, and observations on blood protein regeneration during reduced or zero protein intake have played a historically important role in the formulation of the concept of the dynamic protein equilibrium.1 (3) The anemia of protein deficiency is reversed by feeding of a complete protein.6 During the early phase of realimentation, hemoglobin formation takes precedence over the production of plasma protein.7,8

The protein-deprived animal thus appears to have a mechanism whereby its protein pool is sparingly used in the physiologic replacement of red cells, but in the presence of anemia or after repletion with protein, red cell synthesis seems to occupy a priority position in the utilization of amino acids. Little is known on the regulatory mechanism involved, and the present investigation seeks answers to the following questions: (1) Is the anemia of protein starvation directly caused by a shortage in protein precursors which serve as the building material in red cell formation, or is the erythropoietin regulation involved? (2) What mechanism enables the erythroid tissue to take precedence in the utilization of amino acids after realimentation of an undernourished animal?
ished animal? (3) Is the regeneration of red cells after bleeding mediated by erythropoietin, and if so, what is the quantitative relation between erythropoietin level and resulting rate of erythropoiesis in the face of protein deficiency? The first two of these questions have a bearing on the regulation of protein anabolism in general because erythropoiesis in the protein-deprived organism can serve as a model for study of the significance of primitive control mechanisms—in this case, lowered substrate concentration—in comparison with that of a specific regulatory hormone. The third question is related to an interrelationship of erythropoietin and responsiveness of the erythroid marrow. Rate-limiting processes or conditions may cause an inhibition of erythropoiesis which is relative as far as the effect of erythropoietin is concerned. The latter would still accelerate erythropoiesis, but the resulting rate would be a function of responsiveness and of erythropoietin level.

**METHODS**

Female Holtzman rats of 180 ± 10 Gm. of body weight were used in groups of four, and all data refer to mean and range of such a group. Purina rat chow was given to the normal diet groups. The protein starvation groups received ad libitum a Protein Depletion Diet (Nutritional Biochemical Corp.) containing 84 per cent dextrin, 9 per cent corn oil, 2 per cent agar, 1 per cent cod liver oil and 4 per cent vitamin-salt mixture. The latter included ferrous sulphate in the amount of approximately 1 mg. per 1 Gm. of diet. In addition, 1 mg. Fe (Proferrin) was given to each rat intramuscularly 7 days prior to radioiron administration. For re-proteinization, each rat received 2 Gm. of enzymatically hydrolyzed lactalbumin (Albamin) by stomach tube and thereafter Purina rat chow.

Fe$^{59}$ incorporation and red cell mass (Cr$^{51}$) were measured simultaneously as described.$^9$ The amount of iron injected intravenously was in the order of 0.05 μg. per rat. The iron incorporation was calculated from the radioactivity found in 0.1 ml. of red cells (heart blood) after 24 hours and from the measured red cell mass. Hematocrits were done in duplicate by micro method.

Erythropoietin was obtained according to Borsook’s method$^{10}$ from plasma of rabbits made rapidly anemic by bleeding plus injection of phenylhydrazine. The erythropoietin activity of the extract was measured against Erythropoietin Standard A (N.I.H. Bethesda and N.R.I. London) and the administered doses of erythropoietin are expressed as units of this standard. The nitrogen content of the extract equalled approximately 0.6 mg. N per unit erythropoietin.

**RESULTS**

(1) Effect of Acute Protein Deprivation and Realimentation on Fe$^{59}$ Incorporation

Protein deprivation resulted in a rapid depression of Fe$^{59}$ incorporation as seen in figure 1. Radioiron, injected 48 hours after starting the diet, was incorporated at less than one-half of the normal rate. The depression reached its maximum within 6 days, and the Fe$^{59}$ incorporation remained at the same level thereafter until the 3rd week, when small increases were noted. Table 1 shows iron incorporations and reticulocytes measured during a protein deprivation of 5 weeks’ duration. The animals remained in good general condition throughout this period, but lost about 30 per cent in body weight in spite of their free access to the protein-free food.
Fig. 1.—Body weight and 24-hour Fe\(^{59}\) incorporation during acute protein starvation and realimentation. Mean and range of four rats in each group.

The severity of erythropoietic depression varied greatly with the pre-diet body weight as seen in figure 2. The time course of changes in Fe\(^{59}\) incorporation was similar in the three weight groups and followed a strikingly exponential curve, especially in the 100 Gm. weight group whose Fe\(^{59}\) incorporation dropped from 34 to 0.5 per cent.

Protein feeding, initiated by giving 2 Gm. of lactalbumin by stomach tube, rapidly reversed the erythropoietic depression. Radioiron, injected 48 hours after the protein, was already incorporated 10 times faster than during the protein deprivation period, and the recovery was complete within 3 days after realimentation.

(2) Red Cell Mass during Prolonged Protein Deprivation

Nine groups of rats of 180 ± 5 Gm. of body weight were used in the experiment illustrated in figure 3. The red cell mass was measured in two of these groups before placing the animals on a protein-free diet, and in the remaining groups after periods of protein deprivation as indicated. The observed rate of red cell formation was small (table 1) during the protein deprivation period and can be neglected. The change in red cell mass thus reflects the rate of red cell destruction and its linear decline is compatible with a removal by senescence. Extrapolation of the curve indicates a red cell life span in the order of 70 days which is within the range of from 50 to 100 days reported in normal rats.\(^{11}\) The increasing severity of the anemia during longer protein deprivation thus is not related to any progressive deficiency in protein precursors. Erythropoiesis becomes depressed within a few days of protein
deprivation, and the progressive decline in red cell mass is the cumulative result of the removal of senescent cells in the absence of any significant replacement.

(3) Effect of Erythropoietin Administration on Fe\textsuperscript{59} Incorporation and Red Cell Mass in Protein-deprived Rats

Figure 4 shows the results of erythropoietin administration on the Fe\textsuperscript{59} incorporation in rats which had been kept on a protein-free diet for 10 days. The erythropoietin was injected in two divided doses with a 24-hour interval, and the radioiron was administered 48 hours after the first dose. The dose-response curve is of a similar sigmoid shape as those reported in polycythemic or starved rats.\textsuperscript{12,13} The duration of protein deprivation had no significant effect upon the response. Two units of erythropoietin given to groups of rats after a protein deprivation ranging from 1 to 5 weeks elicited nearly the same increase in Fe\textsuperscript{59} incorporation and reticulocytes (table 1).

The experiments described here were concerned with an erythropoietin-stimulated erythropoiesis of a few days duration. The question whether an accelerated or normal erythropoiesis can be sustained by erythropoietin administration over prolonged periods of protein starvation was studied in two companion groups of rats employed in the measurements of the red cell mass (fig. 3). One of these groups received 0.5 and the other 1.3 units of erythropoietin daily from the 4th to the 26th day of protein deprivation. Their red cell mass, measured on the 28th day, was markedly different from that of the untreated groups (fig. 3). The daily injection of 1.3 units of erythropoietin not only prevented the decline in red cell mass during protein starvation but effected a small increase (+11 per cent) over the baseline. The body weights on the 28th day were 133 Gm. in the untreated group (loss of 28 per cent), 131 Gm. in the group injected with 0.5 units erythropoietin (loss of 30 per cent) and 135 Gm. in the group receiving 1.3 units erythropoietin (loss of 27 per cent). The results indicate that the protein-starved rat, provided with sufficient erythropoietin, manufactures red cells at a normal rate over prolonged periods of negative nitrogen balance and in spite of a serious loss in body weight.

**DISCUSSION**

The presented erythроkinetic data reveal a notable lack of correlation between duration of protein starvation and severity of erythropoietic depression.
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Fig. 2.—Effect of protein deprivation on 24-hour Fe\textsuperscript{59} incorporation in rats of 300, 200 and 100 Gm. body weight.

The experiments were limited to periods of 5 weeks of protein deprivation and no attempt was made to induce additional protein losses by phlebotomies because it was the aim of this investigation to study physiologic regulations during protein deficiency rather than the effects of extreme depletion. The withholding of protein from the food resulted in a rapid change in the iron incorporation which dropped to a minimum within a few days and remained at that level until small increases rather than further decreases were noted during later stages of protein deprivation. Erythropoietin injected after various periods of protein deprivation, ranging from 1 to 5 weeks, caused nearly identical increments in red cell formation, indicating that the necessary protein precursors were equally available throughout this period. The amount of nitrogen used in red cell formation is small in comparison with the animal's total nitrogen requirements. Maintenance of body weight, for instance, requires a protein intake of 60 mg. N per 100 Gm.,\textsuperscript{14} whereas a normal erythropoiesis involves synthesis of protein in the order of 2 mg. N per 100 Gm. body weight per day. The body nitrogen reserve, on the other hand, is relatively large. It resides predominantly in cytoplasmic protein,\textsuperscript{15,16} and the breakdown of this protein replenishes amino acids which are used in anabolic processes during periods of zero protein intake. The maintenance of a normal red cell mass in erythropoietin-injected rats during 4 weeks of protein starvation indicates that an adequate supply of the marrow with amino acids was indeed maintained. A progressive exhaustion of an essential building material
Fig. 3.—Red cell mass during protein deprivation. The upper two curves refer to groups of rats which received daily injections of 1.3 and 0.5 units of erythropoietin.

can therefore not be considered as the primary cause of the erythropoietic depression during protein starvation, and its mechanism must be sought in processes which govern the proliferation of erythroid cells. Two possibilities are considered: (1) a diminished erythropoietin formation, and (2) a retardation of protein synthesis in erythroid cells due to a suboptimal concentration of substrate.

The availability of protein precursors is related to the size of the protein pool and must be distinguished from possible effects of a subnormal concentration of these precursors. Protein starvation results in a widespread depression of protein synthesis. Cessation of growth, decrease in cytoplasmic mass of parenchymal organs, diminished spermatogenesis or plasma protein formation and granulocytopenia are well-documented examples. A suboptimal substrate concentration is thought to be the common cause, retarding protein synthesis either due to a dependence of the rate of enzymatic reactions on substrate concentration, or by more intricate effects of intracellular amino acid concentrations on enzyme formation or activity.

The demonstrated prevention of the anemia of protein starvation by erythropoietin administration would seem to favor a diminished erythropoietin formation as its chief cause, but it does not eliminate the rate-limiting effect of lowered substrate concentration as a major factor. The site and mode of action of erythropoietin are not exactly known, but since it causes the formation, per unit of time, of a greater number of erythrocytes representing a larger cytoplasmic mass, one is justified to assume that it enhances the rate of anabolic processes in erythroid elements at some stage of their development. Its accelerating effect could thus conceivably counteract a retardation in protein synthesis caused by rate-limiting effects of lowered substrate con-
Fig. 4.—Effect of erythropoietin injection on Fe$^{59}$ incorporation in protein-deprived rats. Mean and range of four rats in each group.

centration; in other words higher erythropoietin levels would mask these retarding effects. The rate of erythropoiesis under these circumstances would be proportional to erythropoietin level and erythroid responsiveness. Measurements on the first two of these parameters would permit an assessment of the third, the erythroid responsiveness, and would thus reveal any possible rate-limiting effect of lowered substrate concentration. The results of such experiments are presented in the accompanying paper.

**SUMMARY**

(1) Protein deprivation in rats resulted in a rapid depression of iron incorporation. The depression reached its maximum within 6 days. Realimentation with protein was followed within 3 days by a return of iron incorporation to normal values.

(2) Red cell mass declined during protein starvation in a linear fashion, indicating a removal of senescent red cells after a life span of 70 days. The increasing severity of the anemia of protein starvation is the cumulative result of this removal in the absence of any significant replacement.

(3) Daily injections of 1.3 units of erythropoietin prevented a decrease in red cell mass over an observation period of 28 days of protein starvation.

(4) Diminished erythropoietin formation or retardation of protein synthesis in erythroid precursors due to lowered substrate concentration are considered as possible causes of erythropoietic depression.

**SUMMARIO IN INTERLINGUA**

1. Privation de proteina in rattos resultava in un rapide depression del incorporation de ferro. Le depression attingeva su maximo intra sex dies. Re-
alimentation con proteína eseva sequite intra tres dies per un retorno del incorporation de ferro a valores normal.

2. Le massa erythrocytic declinava durante le privation de proteina linearmente. Isto indica un elimination de senescente erythrocytos post un longevitate de 70 dies. Le crescente severitate del anemia de privation de proteina es le resultato cumulative de iste elimination in le absentia de un significative reimplacamento.

3. Injectiones diurne de 1,3 unitates de erythropoietina preveniva un declino in le massa erythrocytic in le curso de un periodo de observation de 28 dies de privation de proteina.

4. Un reducete formation de erythropoietina o un retardo del synthese de proteina in precursores erythroide (in consequentia de un declino del concentration del substrato) es considerate como causas possibile del depression erythropoietic.

REFERENCES

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