The Effect of Acute Protein Deprivation upon Erythropoiesis in Rats

By W. F. Bethard, R. W. Wissler, J. S. Thompson, M. A. Schroeder and M. J. Robson

The mechanism of anemia associated with malignant disease is not completely understood. Excessive hemolysis and hemorrhage are real factors, yet anemia may be severe without either condition being marked. Bone marrow, in such cases, is occasionally replaced by neoplastic tissue, but this does not occur frequently enough to explain all "myelophthisic" anemias. With the observation that hypofunction or architecturally normal bone marrow does occur, further explanation becomes necessary. Because there is often a clinical correlation between the presence of anemia and aberrations in protein metabolism of patients having neoplastic diseases, it is possible that protein deficiency resulting either from dietary lack or from preferential protein utilization by tumor may play an important role. Experiments with laboratory animals were designed to initiate new investigations of the role of protein in erythropoiesis.

Recognition of the importance of dietary protein in red cell production is not recent. Attempts to control polycythemia vera by diet were made in 1936 by Kleiner. Whipple began his classical experiments in dynamics of protein metabolism and hemoglobin formation in 1918 when he described continued but reduced hemoglobin production during starvation in "standard anemic dogs." Not until 1923, however, did he differentiate between iron and protein as limiting factors in hemoglobin synthesis. In 1922, Jencks demonstrated that the regeneration of blood in rats made anemic by bleeding could be augmented by adequate dietary protein and inhibited by protein deprivation. This was confirmed by McCay in 1928. Orten and Smith reported in 1937 the appearance of hypochromic anemia in rats subjected to chronic protein deficiency. In the same year Pearson, Elvehjem, and Hart concluded that hemoglobin formation in rats was more vital than growth and hence was precedent in protein utilization. In 1939, Hahn and Whipple stated that reduced protein intake in dogs leads to economic utilization of tissue protein for continued production of hemoglobin, and by 1942 they had determined that "standard anemic dogs" on basal low-protein diets could make 40 to 50 Gm. of hemoglobin per week for several weeks in the absence of infection. Furthermore, if protein was fed to anemic and hypoproteinemic
dogs, hemoglobin formation invariably took precedence over production of plasma protein. Anemia secondary to protein deficiency has been shown to be reversible. In 1945 Metcoff, Favour, and Stare reported a comprehensive series of experiments on erythropoiesis in protein-deficient rats. They were the first to present data on changes in plasma volume and red cell volume following acute protein deprivation, and they postulated a dynamic balance between tissue and circulating proteins that could shift in either direction as the need arose.

There is much additional information pertaining to the efficacy of various foods and amino acids in protein repletion. In general, most conclusions favor sustained erythropoiesis at the expense of tissue-protein stores. The availability of radioiron and its use in the quantitation of erythrocyte formation now provides a potentially more precise experimental method than has heretofore been available. Further evaluation of the relationships between dietary protein and red cell formation by means of this tracer technic was considered desirable.

**Materials and Methods**

*First Uptake Experiment:* Young adult male Sprague-Dawley rats, weighing from 164 to 216 Gm. (mean = 194 Gm.) were divided randomly into two groups of 14 and 21 animals respectively. Half of the first group was maintained on Purina Lab Chow ad libitum, and the other half received daily 15 Gm. of a synthetic, nutritionally adequate diet designated 4C (table 1). All members of the second group received daily 15 Gm. of a synthetic, low-protein diet (4E) that was devised by Cannon et al. Water was not restricted. On the 35th day of the experiment, protein repletion of the second group (protein-depleted) was begun by substituting the 4C for the 4E diet. The experimental synthetic diets were isocaloric. At each of the seven sampling periods 1 rat from each control-diet group and 3 rats from the low-protein diet group were used. All rats were weighed and examined for signs of infection at weekly intervals during the experimental period.

Erythrocyte radioiron uptake was determined on the 1st, 15th, 25th, 35th, 50th, 60th, and 70th days of the experiment. This made it possible to evaluate incorporation of iron into newly-formed erythrocytes at three intervals during protein depletion and at three

| Table 1—Composition of Diets (Gm. per 100 Gm.) |
|-------|-------|-------|-------|
|       | 4E    | 4C    | Low Calorie A | Low Calorie B |
| Dextrin | 69    | 48.7  | 9.2         | 5.9         |
| Corn Oil | 4     | 4     | 3.7         | 1.0         |
| Ruffex | 5     | 5     | 9.2         | 10.0        |
| Jones and Foster Salt Mix | 4 | 4 | 7.4 | 7.9 |
| Multi-vitamin Mix | 2 | 2 | 3.7 | 4.0 |
| Casein | 0     | 22    | 40.0        | 43.0        |
| Water  | 15.8  | 14.1  | 26.0        | 27.0        |
| Oleum Percom. | 0.3 drops | 0.3 drops | 0.3 drops | 0.3 drops |
| Choline Chloride | 0.182 | 0.182 | 0.182 | 0.182 |

Low calorie diets A and B contained respectively approximately 2.4 and 2.2 calories per Gm.
intervals during repletion. Radioiron* (0.3 microcuries of Fe²⁺ having a specific activity of approximately 0.2 mc. per mg.) was injected intravenously into the animals to be sampled five days prior to their sacrifice. Five days after injection, and at the intervals designated above, hemoglobin, hematocrit, and red cell volume measurements were made on these animals. Radiophosphorous-tagged erythrocytes were used for the latter. An aliquot of blood was drawn by cardiac puncture for Fe²⁺ determination, and from this value plus that of the red cell volume, the per cent of injected Fe²⁺ contained in red cells was calculated. Each sample was ashed and electroplated according to the method of Peacock and Evans as modified by Berlin. A Tracerlab TCG-2 Geiger-Müller tube was used to determine the radioactivity of the samples.

Second Uptake Experiment: When results from the first uptake experiment were examined, it became apparent that confirmation was required. Accordingly, the work was repeated using a greater number of rats and more frequent sampling periods. Effects of protein repletion were omitted. Two new experimental groups were added, however, to include animals given high-protein, low-caloric diets to insure weight loss comparable to that observed in protein-depleted rats. The diets are given in table 1. Blood samples were taken just before special diets were begun and at five-day intervals over a 35 day period thereafter. Enough animals were used so that the number for each sampling period was as follows: 4 on diet 4E, 2 on diet 4C, and 2 on Purina Lab Chow. The two low-caloric, adequate protein groups, consisting of 4 animals each, were sampled on the 35th day. The determinations made, as well as the amount of Fe²⁺ injected and technical methods used were the same as those described for the first uptake experiment.

RESULTS

Except for weight and blood volume, results for rats fed Purina Lab Chow and those fed diet 4C were comparable and are thus represented as a single group (solid line) in figures 2, 3, and 5. As can be seen in figure 1, protein-depleted rats lost 25 to 30 per cent of their body weight in 35 days. The loss was consistent in both the first and second experiments. The rate of loss was greater at the beginning of protein deprivation than later. Upon repletion, weight loss was promptly supplanted by weight gain and at a rate slightly greater than in the normal rats. Animals fed 15 Gm. per day of an adequate protein, synthetic diet (4C) gained weight, but at a rate considerably less than rats fed Chow ad libitum.

Hemoglobin and hematocrit values of control and protein-depleted animals are shown in figures 2 and 3. In all of the experiments, hemoglobin and hematocrit values were higher in protein-depleted animals than in the controls until the 20th to 25th day. At that time, however, the values dropped below the normal level and remained low. With protein repletion they increased promptly to a point just below normal. Examination of red cell and plasma volumes, however (fig. 4), indicates that initially high hemoglobin and hematocrit results were relative and were due to disproportionate decreases in plasma and red cell volumes after protein deprivation was begun. On an absolute basis, red cell volume dropped promptly in animals receiving either the 4E or 4C diets, but the drop was of greater magnitude and of longer duration in the former group. Except for the 4E group, blood volumes tended to increase with time. This correlated well with the rapid growth of the

*Supplied by Oak Ridge National Laboratory and prepared by neutron bombardment of electromagnetically enriched Fe²⁺.
young rats, and paralleled their weight gain. With protein deprivation, plasma volumes tended to drop rapidly at first and then to level off. Red cell volumes dropped less rapidly but more constantly.

Since iron does not directly enter mature erythrocytes, the rate at which tagged iron enters the peripheral red cell volume ostensibly represents the rate at which new red cells are being formed. It would be advantageous to measure circulating radioiron constantly to follow changes in erythropoietic rate. Practically, however, it is necessary to select arbitrarily an optimum interval and to consider the associated rate as an integral of all intervening rates. Rather than use the maximum radioiron ultimately appearing in the circulation as an indication of erythropoietic rate, a constant interval of five days after radioiron injection was selected. Previous experience had shown that total circulating radioiron was only slightly less than maximum at five days and that the small difference was consistent.
It was assumed that all animals were healthy and had normal iron stores and normal bone marrow function at the beginning of the experiment. As can be seen in figure 5, erythropoiesis decreased rapidly when protein deprivation was instituted. Within 10 days, protein-deprived animals could utilize only 5 per cent of the injected radioiron for red cell production (in the arbitrary five-day interval) as compared with 71 per cent utilization by normal rats (including those on diet 4C). Subsequent to this rapid drop there was a short rise followed by a prompt fall almost to zero, and then another rise. This cyclic pattern was observed in the two independently performed experiments. Results of both experiments were comparable, except for slight differences in time relationships. The data from the two groups that received low-calorie, adequate protein diets are also shown in figure 5.
FIG. 3.—Average hematocrit values of normal (solid line) and protein-deficient (dotted line) rats.

After 35 days of such diets, the rats could utilize 34 to 37 per cent of injected radioiron. These values are somewhat lower than normal, but significantly greater than those for protein-depleted rats.

DISCUSSION

Anemia, as indicated by conventional measures of peripheral hemoglobin and corpuscular concentration, has been observed previously in rats fed a low-protein diet.6,7,15 Metcoff, Favour, and Stare11 described hemoconcentration and reduction in total circulating hemoglobin following acute protein depletion in rats; and similar data were obtained in the experiments discussed in this paper. The mechanism of the rapid drop in plasma volume, hence total blood volume, shortly after removal of protein from the diet is not clear. Hypoproteinemia, with resultant transudation of fluid from the vascular system, is a theoretical cause, but total circulating serum proteins do not decline within the first 10 days after institution of diet 4E.12,16 Plasma volume decreased rapidly at first and then stabilized at a lower level in contrast to changes in total circulating serum protein that began after 10 days and then progressed rapidly.16

Animals fed diet 4C gained weight slowly because they were limited to 15 Gm. daily (fig. 1). Erythropoiesis in these animals remained normal despite
the fact that red cell and plasma volumes dropped slightly before their eventual rise.

The decline in erythropoietic rate following protein deprivation, as measured by the methods described, was unexpectedly fast. It appeared too early to be the result of depletion of tissue protein stores\textsuperscript{16}. Inability to synthesize new protein is an unlikely cause inasmuch as antibody formation in rats may continue for 17 to 28 days after acute protein deficiency\textsuperscript{16}. Apparently,
it is the result of an attempt by the organism to utilize available protein in accordance with a priority system that provides maximum biologic efficiency under the stress imposed. In Whipple's experiments on "standard anemic dogs," anemia was achieved by the removal of erythrocytes. The rats used in this series of experiments were not made anemic, and measurements were begun when the circulating red cell volume was normal. The apparent difference in results may be explained by the fact that erythropoiesis was
stimulated by anemia in one case but not in the other. It seems reasonable to suspect that protein-depleted animals may have a mechanism whereby available reserve protein may be utilized either to maintain tissue protein in the absence of anemia, or to manufacture hemoglobin when anemia is present. Sporadic spurts in erythropoiesis, as represented by the cyclic pattern displayed in the two independently performed experiments, support the idea of dynamic balance between tissue and blood proteins. That this balance can be overcome is indicated by the observation that protein deficient rats become polycythemic when given cobalt, just as do normal rats.\textsuperscript{17} Perhaps it is not pure coincidence that, after an initial rapid fall, the amount of iron utilized for erythropoiesis increased, and that this occurred at approximately the same time that the hemoglobin and hematocrit values fell below control levels (figs. 2 and 3). The rate of erythropoiesis fluctuated without any marked alteration in red cell volume or total blood volume (fig. 5). This would suggest that the concentration of hemoglobin within the vascular system is more important for homeostasis than the total amount of hemoglobin contained therein. Failure to sustain an increased erythropoietic rate, even though hemoglobin concentration did not rise, may have been a manifestation of severe protein depletion. It should be interesting to determine whether or not other erythropoietic stimuli, such as hypoxia or phenylhydrazine hemolysis, alter the results of the present experiments. Elucidation of the mechanism or mechanisms governing protein utilization is of great importance. It is conceivable that preferential utilization of protein by rapidly growing tumors in debilitated patients could result in markedly decreased erythropoiesis in a way that is analogous to that demonstrated in protein-deficient rats.

**Summary**

1. Hemoglobin concentration, blood volume, and erythrocyte radioiron uptake were measured in rats subjected to acute protein deficiency.
2. Removal of protein from the diet was followed promptly by hemoconcentration, diminution in blood volume, and drastic reduction in erythropoiesis. These changes were reversible, after 35 days, upon addition of protein to the diet.
3. Protein intake is more essential for maintenance of normal erythropoiesis than is total caloric intake.
4. The data suggest that hemoglobin concentration within the vascular system is more important than red cell volume in regulating erythropoietic rate.

**Summario in Interlingua**

1. Le concentration de hemoglobina, le volumine de sanguine, e le acceptation de ferro radioactive per le erythrocytos eseva mesurate in rattos subjicite a acute carentia de proteina.
2. Le elimination de proteina ab le dieta eseva segue promptemente per hemoconcentration, reduction del volumine de sanguine, e un drastic diminution del erythropoiese. Iste alterationes eseva reversibile, post 35 dies, per le addition de proteina al dieta.
3. Le ingestion de proteina es plus importante pro le mantenentia de un erythropoiese normal que le ingestion total de calorias.

4. Le datos suggereque le concentration de hemoglobina intra le systema vascular es plus importante que le volume erythrocytic pro le regulation del intensitate erythropoietic.

REFERENCES

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