Cali-Harvard Nutrition Project

III. The Erythroid Atrophy of Severe Protein Deficiency in Monkeys

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IT HAS been postulated that the anemia of children with severe protein malnutrition (kwashiorkor and marasmus) is due primarily to a defect in the production of red cell precursors. Erythroid hypoplasia was a consistent finding. After initiating protein feeding, there was an increased production of normoblasts, and, in most cases, erythroid hyperplasia occurred. This was also observed in those patients whose bone marrow smears demonstrated normal concentrations of normoblasts on admission. In the latter case, the anemia was attributed to a functional impairment in the development of red cell precursors.[1]

Rats have been shown to have erythroid hypoplasia when fed a protein-free diet.[2] However, no serial studies on the changes in bone marrow structure as a result of protein deficiency and subsequent refeeding have been carried out. This is particularly true of the primate. In an extension of our own studies with children with severe protein malnutrition, the following experiments were carried out:

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MATERIALS AND METHODS

Five young monkeys (Cebus fatuella) weighing approximately 1 kg. (range 990 to 1,250 gm.) were placed in individual cages and fed *ad libitum*. The protein-free diet consisted of the following constituents (in grams per 100 grams): glucose 85.49, soy bean oil 9.0, choline chloride 0.3, ascorbic acid 0.05, cod liver oil 1.0, salts IV[3] 4.0 and a vitamin mixture 0.154. The vitamin mixture supplied the following vitamins, in milligrams per kilogram of diet: thiamine hydrochloride 4, riboflavin 8, pyridoxine hydrochloride 4, calcium pantothenate 25, niacin 40, pteroylglutamic (folic acid) 1 and biotin 0.2.

Hemoglobin concentration was determined by the cyanmethemoglobin method and the hematocrit by the capillary-tube method. Less than 10 ml. of venous blood was drawn from each monkey during the time of the experiment. Bone marrow aspirates were obtained from the upper part of the tibia, smeared on cover slips and stained with Wright's stain. Three hundred nucleated cells were counted, noting the number of normoblasts, total granulocytes and lymphocytes. The relative proportions of basophilic, polychromatic and orthochromatic normoblasts were also noted.

RESULTS AND COMMENTS

From an average value of 20 per cent, the normoblast count fell to 11 per cent after one week of feeding with the protein-free diet (Fig. 1). After a slight tendency for the normoblast count to rise during the next three

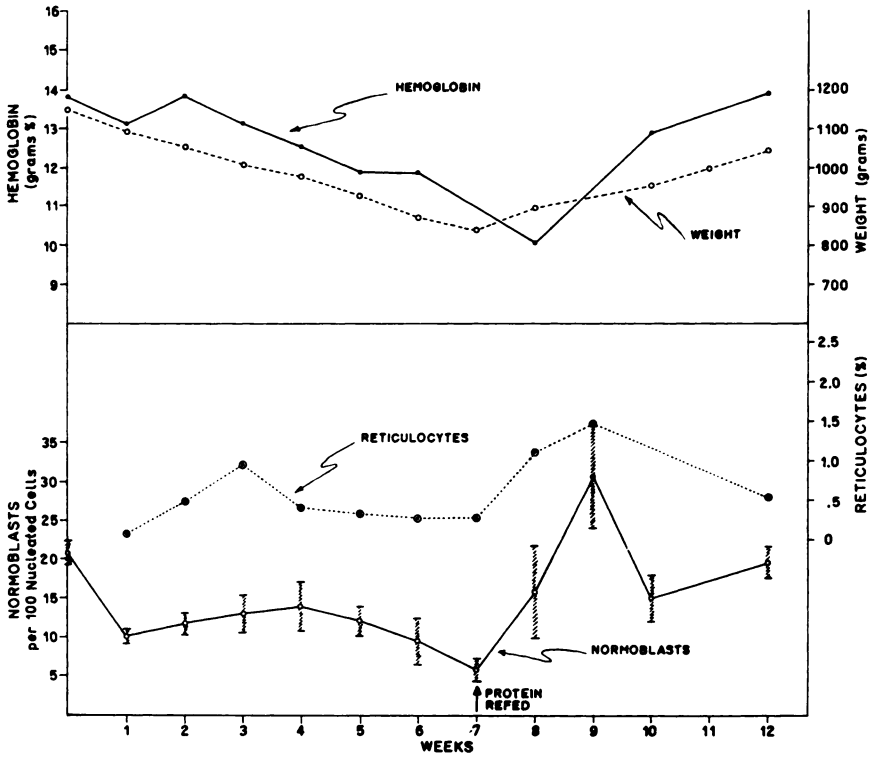

FIG. 1. Changes in normoblast and reticulocyte counts, hemoglobin concentration and weight of monkeys fed a protein-free diet. Crosshatched lines indicate standard error of the mean value obtained from five monkeys.

weeks, it reached its lowest value of 6 per cent at the end of seven weeks. At this time, protein was added as 10 per cent of the diet at the expense of glucose in the form of devitaminized casein. During the following two weeks, there was a marked increase in the number of normoblasts, reaching an average level of 31 per cent, which is significantly higher than the control value. Shortly thereafter, a decline to normal count was obtained. The percentage of granulocytes remained relatively constant; the changes in normoblast counts were made up by reciprocal changes in the number of lymphocytes. No consistent modifications in the distribution of the different types of normoblasts were observed throughout the experiment.

The heaviest monkey of the group, behaving somewhat differently, maintained relatively constant normoblast counts while ingesting the protein-free diet. A value of 14 per cent was observed at the end of seven weeks, in contrast to 1 to 8 per cent in the other four monkeys. Yet, two weeks after refeeding, this animal had the most pronounced hyperplasia, a normoblast count of 54 per cent in contrast to the 22 to 40 per cent observed in the other animals. This monkey was also the quickest to recover from the anemia, and the erythroid hyperplasia which developed with refeeding suggests that he had a functional impairment in the production of the red cell precursors.

The reticulocyte counts showed minimal variations (Fig. 1), and the significance of weekly counts is questionable. However, they tended to reflect the changes in normoblast counts.

It is uncertain that the total amounts of granulocytes decreased as only relative proportions of bone marrow cells were counted. However, granulocytes are evidently more resistant than normoblasts to protein lack,

although the former turn over much more rapidly than the latter. This paradox perhaps can be explained in part by the fact that many more red cells than granulocytes are produced per unit time.

In the upper part of Figure 1, the changes in weight and hemoglobin concentrations during the course of the experiment are seen. The parallelism between the two curves may be fortuitous. The hematocrit values are not shown, but they followed the hemoglobin values and, therefore, the mean corpuscular hemoglobin concentration remained always within normal limits. From an average control value of 13.8 gm. per 100 ml., the hemoglobin concentration had fallen to 11.9 gm. at the end of six weeks. A blood sample was not taken on the seventh week, for by this time, the animals were in a rather poor condition. As expected,[3,4] the lowest level in hemoglobin concentration (10.0 gm.) occurred after refeeding with protein followed by recovery to control levels.

In severe protein deficiency there is a decreased total blood volume. Therefore, the hemoglobin concentration under such conditions does not give an accurate picture of the true decrease in total circulating hemoglobin.[5] In general, however, a hemoglobin value of less than 8 to 9 gm. per cent noted in protein deficiency or in a child with kwashiorkor or marasmus on admission should make one suspect associated causes for the anemia.[1,3,4] When protein is refed, plasma volume is restored more rapidly than red cell volume, producing the initial misleading decrease in hemoglobin.

Atrophy means wasting away due to lack of nourishment.[6] It is known that several tissues become atrophic with protein deprivation,[7–11] and it is reasonable to believe that the erythroid hypoplasia of protein deprivation represents the atrophy of the erythropoietic tissue. Thus, we have chosen to speak of erythroid atrophy when referring to the erythroid hypoplasia caused by protein malnutrition. It is not implied that only protein malnutrition causes erythroid atrophy. Perhaps, the erythroid hypoplasia of hypothyroidism is also an example of erythroid atrophy, caused by a deficiency of the thyroid hormone.

SUMMARY

Erythroid atrophy developed rapidly in Cebus monkeys when they were fed a protein-free diet. When refed, there was an initial erythroid hyperplasia followed by a return to control normoblast counts. The findings confirm the observations previously made in patients with kwashiorkor and marasmus and support the postulate that the basic mechanism producing the anemia of protein deficiency is an atrophy of the erythropoietic tissue.

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