Clinical signs of anemia in vitamin A-deficient rats[1-3]

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ABSTRACT Young rats weighing 150 g (initial weight) were fed diets sufficient or deficient in vitamin A. Postweaning rats were used in order to retard the rapid onset of vitamin A deficiency. The effects of the deficiency were studied with respect to impairment of hematopoietic function and anemia. Values for hemoglobin and hematocrit provided evidence of anemia before the signs of severe vitamin A deficiency became apparent. These included alopecia, ocular lesions, and low levels of retinol in plasma and liver. At the point where liver stores of vitamin A were virtually depleted, however, estimates for serum iron, hematocrit, and hemoglobin were elevated to control levels. The latter phenomenon appeared to result from hemoconcentration. These data suggest that anemia may be a component of vitamin A deficiency, but might be masked by the dehydration that accompanies severe depletion of vitamin A. *Am. J. Clin. Nutr.* 32: 1439–1444, 1979.

Several lines of evidence suggest that chronic deprivation of vitamin A results in an anemia with some characteristics similar to those accompanying iron deficiency (1–5). Despite low levels of iron in serum, the anemia associated with vitamin A deficiency does not respond to medicinal iron, but is reversed upon repletion with vitamin A (1). It is characterized by a reduction in the mean red cell volume and the mean red cell hemoglobin concentration; however, depending upon the rapidity of onset and the severity of the deficiency, hematocrit values and blood hemoglobin levels may appear to be normal or slightly elevated (6, 7). This somewhat paradoxical relationship, i.e., a normal or elevated hematocrit, may be due to a decrease in the plasma volume that can occur in vitamin A deficiency (1, 7, 8). Alterations in water balance, particularly a decrease in extra-cellular water, is one of the signs of severe vitamin A deficiency in animals (1, 7, 8). This phenomena can lead to hemoconcentration (1, 7); and can be misinterpreted as polycythemia (cf. Reference 1 and 8).

As will be pointed out herein, there are variations in the hemoglobin and hematocrit levels in vitamin A-deficient rats that appear to depend upon the degree of vitamin A deficiency. We will show that a significant depression in hematopoietic function may be observed in the initial stages of vitamin A deficiency. However, at that point where animals become severely deficient, based on almost total depletion of retinol from hepatic stores, the common indices for anemia (hemoglobin, hematocrit, serum iron) do not appear to be abnormal. Although an exact mechanism is not proposed, these data, in addition to our previous observations (1, 7), suggest that when rats are vitamin A deficient there is impaired iron utilization which may result in anemia, but with severe deficiency this anemia is masked.

Materials and methods

Animals and diets

Sprague-Dawley rats (Simonson, Gilroy, Calif.) weighing approximately 150 ± 10 g were used. Each animal was housed in a separate wire cage. They were

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divided into three groups. All three groups were fed a semipurified diet, complete in all essential nutrients except vitamin A (Table 1). Control groups were given retinyl palmitate (6500 IU/kg diet). The control groups were fed the supplemented diet as a single meal, either ad libitum or in amounts no greater than that consumed by the deficient animals. Water was provided ad libitum. The three groups of rats (control ad libitum, group 1; control-food restricted, group 2; and vitamin A-deficient, group 3) were first fed the supplemented diet for 4 days, then six rats from each group were killed in order to obtain base-line data. Thereafter, the rats were weighed

TABLE 1
Formulation of the basal diet

Ingredient	Percentage
Cerelose	61.0
Vitamin free-casein[a]	18.0
Vitamin mixture-sucrose[b,c]	10.0
Salt mixture[d,e]	6.0
Oil (olive oil)	5.0
DL-methionine	0.25

[a] Nutritional Biochemical, Cleveland, Ohio. [b] Provided the following in milligrams per kilogram of diet: cyanocobalamin, 0.2; biotin, 0.5; folic acid, 0.5; menadione, 0.60; thiamin·HCl, 5.0; riboflavin, 5.0; pyridoxine·HCl, 5.0; α-tocopheryl acetate, 45.5; Ca pantothenate, 50.0; niacin, 50.0; *i*-inositol, 100.0; choline chloride, 1000.0; and ergocalciferol, 0.01. [c] The complete diet (control) contained in addition to the vitamins given in footnote *b* retinyl palmitate equivalent to 6500 IU/kg of diet. [d] Provided the following as grams per kilogram of diet: $CaCO_3$, 18; K_2HPO_4, 19.5; $CaHPO_4$, 3.6; NaCl, 10.08; $FeSO_4 \cdot 7H_2O$, 1.5; $MgSO_4 \cdot 7H_2O$, 7.5; KI, 0.015; $ZnCO_3$, 0.048; $CuSO_4 \cdot 5H_2O$, 0.018; and $MnSO_4 \cdot H_2O$, 0.138. [e] Analysis of the basal diet indicated that the total iron content was 35 ± 6.7 mg/kg of diet.

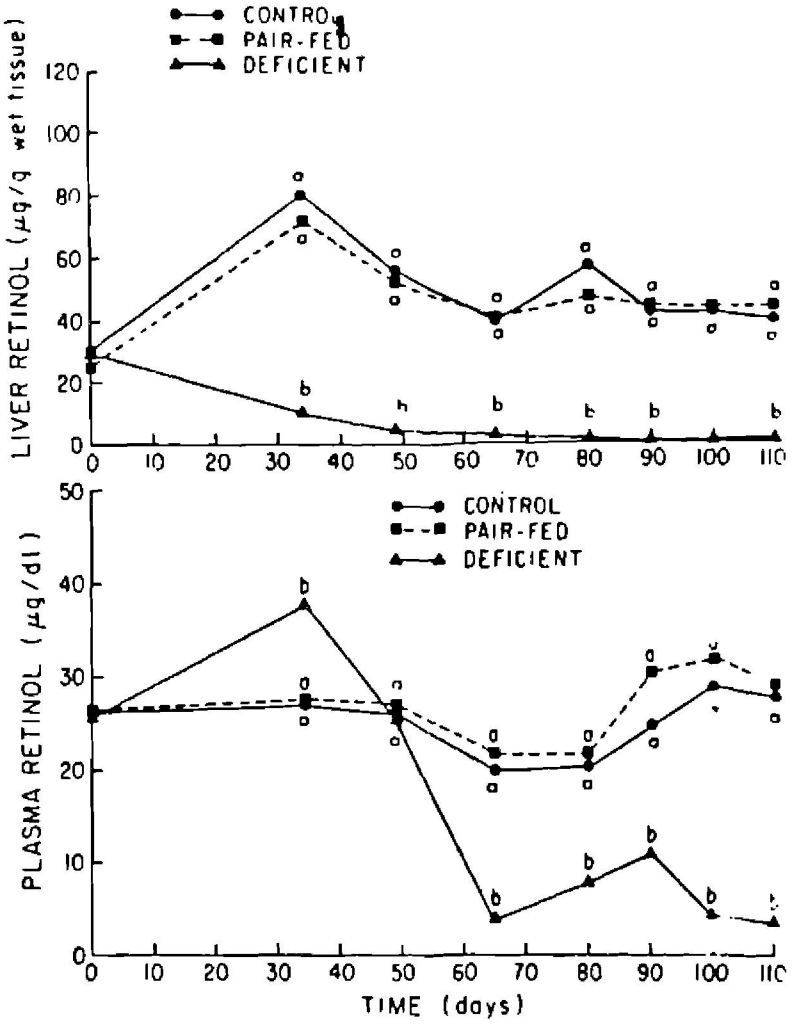

FIG. 1. In the *upper panel* liver retinol values are given for the three groups of rats fed diets either sufficient in vitamin A, deficient in vitamin A, or sufficient in vitamin A but pair-fed in amounts restricted to that of the deficient group. After 30 days, all of the values for the deficient group are significantly different from the two control groups at $P < 0.001$. In the *lower panel*, plasma retinol values for the three groups of rats are given. Values bearing a differing superscript are significantly different at $P < 0.05$.

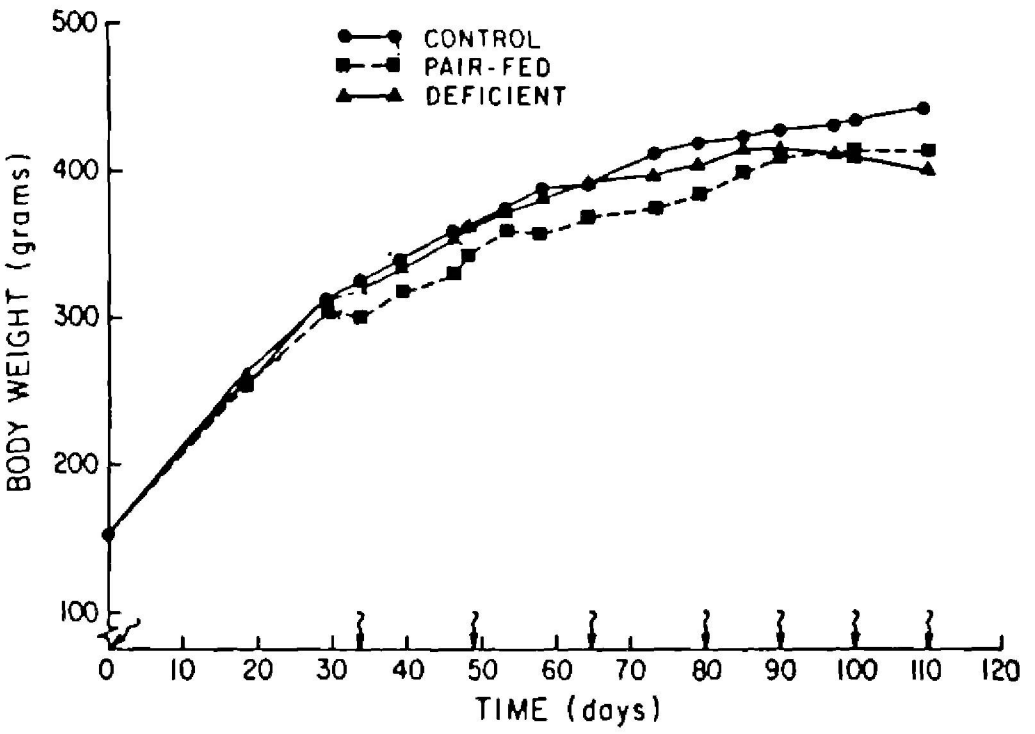

FIG. 2. Growth curves for the three groups of rats during the depletion period. The *arrow* along the *abscissa* indicate times when the animals were killed for the various determinations.

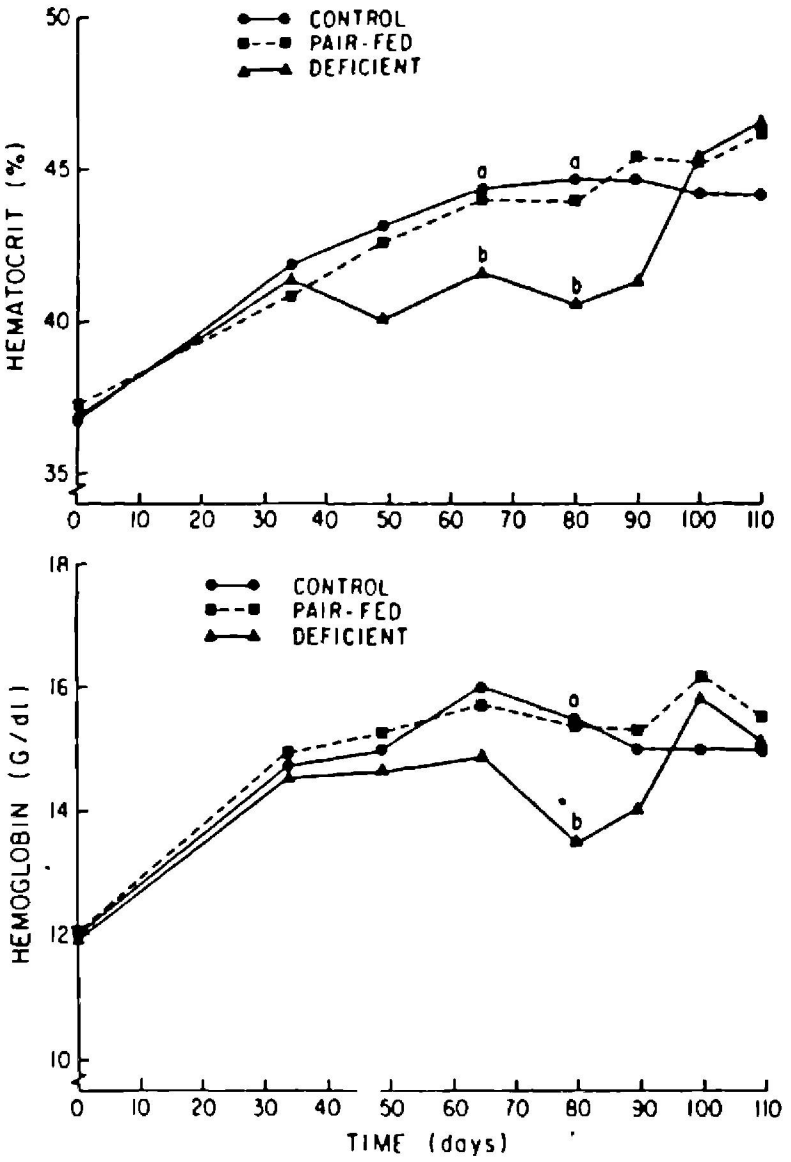

FIG. 3 Changes in the values for hematocrit (*upper panel*) or hemoglobin (*lower panel*) in rats fed diets sufficient or deficient in vitamin A. Each *point* represents the average from six animals. Values bearing a different superscript are significantly different at $P < 0.05$.

periodically and at 34, 49, 65, 80, 90, 100, and 110 days after the initiation of the experiment. At each of these times, six rats from each group were killed by decapitation in preparation for the various determinations.

Determination of iron and hematological indices

Blood was collected in heparinized tubes. The liver and spleen were also removed for the determination of retinol and iron Hematocrit levels, hemoglobin, and red cell counts were estimated using standard hematological techniques (9). The level of retinol in liver and plasma was determined spectrophotometrically (10). The total amount of iron in liver, plasma, and spleen was analyzed by atomic absorption spectrophotometry after "wet ashing" the tissue samples (11). In addition, alterations in red cell morphology were investigated using scanning electron microscopy (12). The red cells were obtained from rats fed their respective diets for 110 days. The cells were fixed immediately in 0.3% gluteraldehyde in preparation for the observations.

Results and discussion

Relatively mature rats (150 ± 10 g) were used intentionally for these studies. One concern was whether perturbations in iron metabolism could be observed without the complications resulting from inanition, which are often observed when weanling rats are fed vitamin A-deficient diets. Also, postweaning rats were used to circumvent some of the early developmental changes observed in rats fed a diet deficient in vitamin A. Evidence that this was accomplished is provided by the fact that even though the vitamin A-deficient rats had markedly reduced hepatic stores of retinol after 30 to 40 days of the deficiency (Fig. 1) a significant reduction in body weight was not observed (Fig. 2). As was expected, the reduction in liver retinol was followed by a rapid reduction in plasma retinol (Fig. 1).

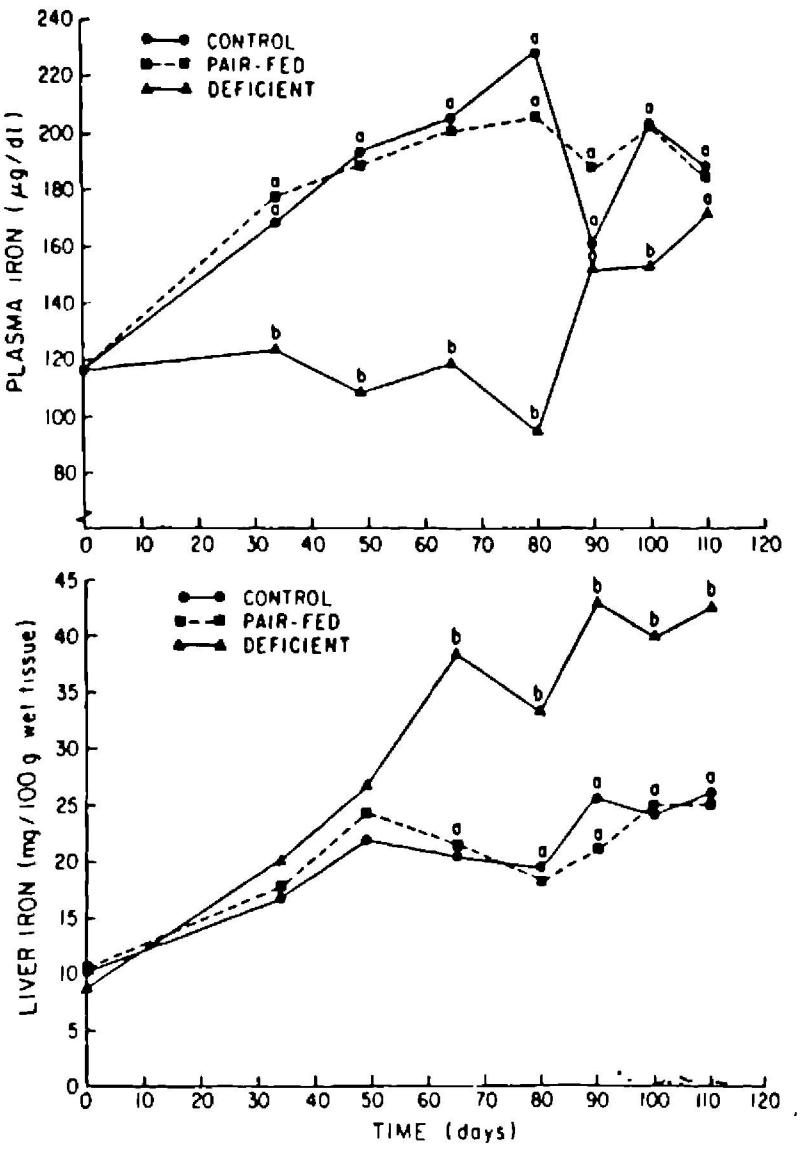

FIG. 4. Values for plasma iron (*upper panel*) and hepatic iron (*lower panel*) for the three groups of rats fed diets either sufficient or deficient in vitamin A. Values bearing a different superscript are significantly different at a level of $P < 0.05$. With growth, there appeared to be a gradual increase in the plasma level of iron in the two control groups. This increase was not observed in the vitamin A-deficient group prior to 80 days. Hepatic iron appeared to be sequested in vitamin A-deficient rats. The increase in hepatic iron appeared to correspond to the onset of anemia observed in vitamin A-deficient rats (cf. Fig. 3).

During the initial period, in which significant reductions in liver and plasma levels of retinol were observed, significant reductions in hematocrit (Fig. 3), hemoglobin (Fig. 3), and plasma iron levels (Fig. 4) were observed in the vitamin A-deficient group compared to the controls. It is important to note, however, that these differences were no longer apparent after 80 to 90 days of deficiency. This phenomena may be related to a reduction in plasma volume. In related studies, we have observed a significant reduction in the plasma volume of vitamin A-deficient rats using the same experimental protocol (7). This point is emphasized because hemoconcentration may be an important reason for the failure of others to observe similar changes in hematological parameters in their vitamin A-deficient animals (6).

That iron metabolism is impaired by vitamin A deficiency is also evident from the increase in liver iron in vitamin A-deficient rats (Fig. 4). In the deficient group, the increase in liver iron appeared to correspond to the decrease in plasma iron (Fig. 4). This suggests that, among several possibilities, the apparent decreased utilization of iron for hemoglobin synthesis may be related to an inability to mobilize iron from hepatic stores. Similar results were obtained in a previous study using weanling rats (1). The degree to which this represents the major factor in the impaired utilization of iron by vitamin A-deficient rats, however, has yet to be fully assessed.

Other indices suggesting impaired hematopoiesis are given in Table 2. The values were obtained from samples taken after 80 days of vitamin A depletion. In addition to significantly lower levels of hemoglobin and hematocrit, both the mean corpuscular volume and mean corpuscular hemoglobin concentration of the red cells were reduced in the vitamin A-deficient rats. At 80 days, the dif-

TABLE 2
Hematological indices of rats in the three experimental groups 80 days after the initiation of study

Hematological indices	Control (1)	Food-restricted (2)	Deficient (3)
Hemoglobin (g/dl)	15.5 ± 0.6[a]	15.4 ± 0.6[a]	13.3 ± 0.4[b]
Hematocrit (%)	44.6 ± 0.8[a]	43.8 ± 1.0[a]	40.7 ± 1.2[b]
Red cell count ($\times 10^6$)	7.7 ± 0.2[a]	7.9 ± 0.3[a]	7.9 ± 0.3[a]
Mean corpuscular volume (μ^3)	58.3 ± 1.6[a]	56.0 ± 1.7[a]	51.7 ± 1.0[b]
Mean corpuscular hemoglobin ($\mu\mu$g)	20.2 ± 0.7[a]	19.7 ± 0.7[a]	17.0 ± 0.6[b]

[a] Mean ± 1 SEM. [b] Significant at $P < 0.05$.

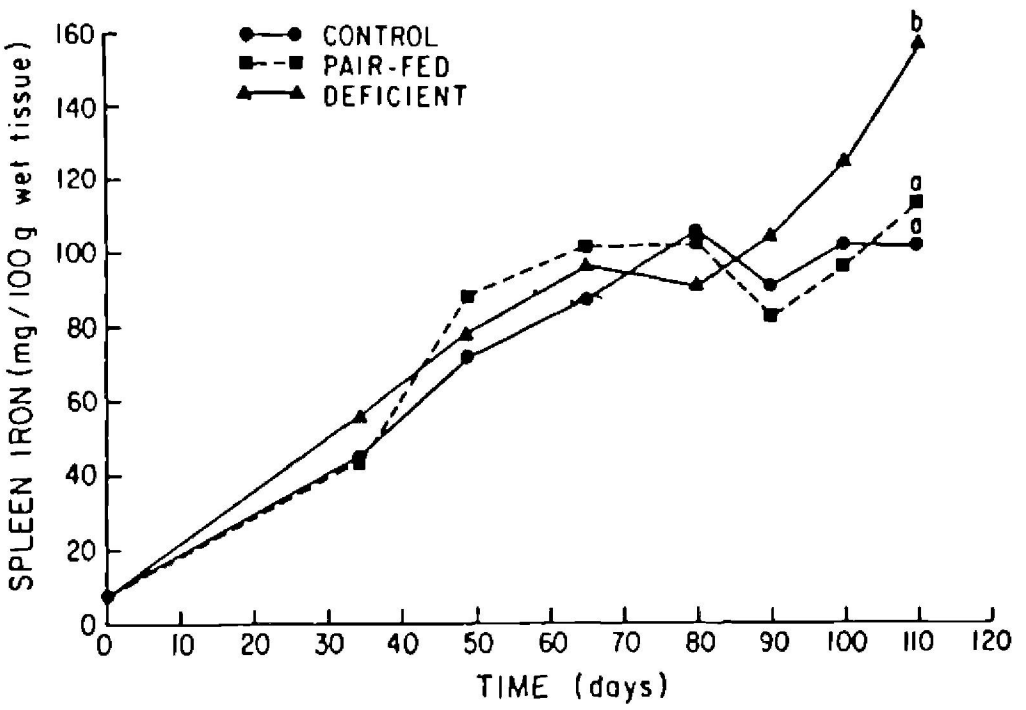

FIG. 5. Values for the total iron concentration in spleen for the three groups of rats during the vitamin A-depletion period. Only the values obtained at 110 days (vitamin A deficient versus the two control groups) were significantly different at $P < 0.05$.

ferences between the vitamin A-deficient rats and the two groups of control rats were statistically significant in all of the four indices used operationally to define impaired hematopoiesis (significantly reduced values for hemoglobin, hematocrit, mean cell volume, and mean cell hemoglobin concentration). Although the vitamin A-deficient rats were not severely anemic, the changes in red cells and iron metabolism were characteristic of the early alterations that occur when iron utilization is impaired. As pointed out above (Fig. 3), when the deficiency was prolonged to more than 80 days, the differences in hematocrit and hemoglobin disappeared. Consequently, the relative severity of vitamin A deficiency appears important with respect to a clear assessment of the hematological defects. Also, in our previous report (1) in which impaired iron utilization was observed in weanling vitamin A-deficient rats, it was noted that the signs of anemia were expressed only if small quantities of vitamin A were fed to the deficient animals at 10-day intervals throughout the depletion period; i.e., a chronic or less acute state of vitamin A deficiency appears important with respect to assessing the anemia.

The values for splenic iron are given in Figure 5. The iron content of spleens increased significantly after 110 days of vitamin A deficiency. This increase occurred near the end of the experimental period when obvious laboratory and visible signs of vitamin A deficiency (mild alopecia, ocular lesions, inflammation of the oral epithelium) were apparent in the rats remaining in the deficient group. It was at this point that alterations in red cell morphology (poikilocytosis) were also observed in the deficient group (Fig. 6). The destruction of poikilocytotic red cells may have resulted in the significant rise in iron, plasma, and spleen. It is of interest, however, that this phenomenon followed the observed signs of anemia and only occurred when the animals became severely deficient. If the initial signs of anemia resulted primarily from a hemolytic process, then it might be expected that plasma and splenic iron would be elevated during or before the period when decreased values for hematocrit and/or hemoglobin are observed.

Although extrapolation from experimental animals to man is often tenuous, relationships

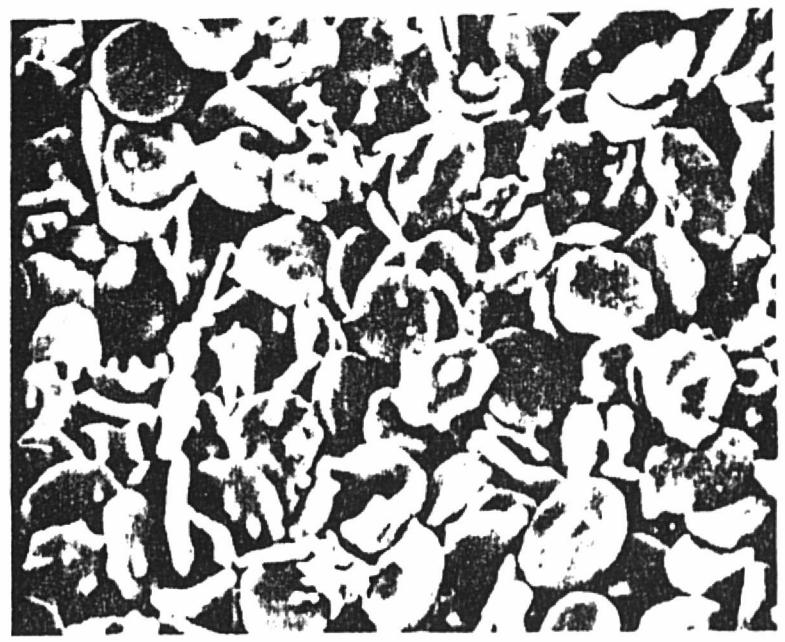

FIG. 6. Scanning electron micrographs of red cells from vitamin A-sufficient (*upper panel*) and vitamin A-deficient (*lower panel*) rats. Poikilocytotic cells are observed throughout the field of red cells from the vitamin A-deficient group. Poikilocytotic red cells were not observed in abnormally high numbers in blood preparations from either of the two control groups of rats.

similar to those reported here have been observed in man. Mejia et al. (3) have presented data that suggest that plasma iron and retinol levels are correlated in younger children, but only when iron intakes are adequate. Mohanram et al. (2) also have reported anemia in vitamin A-deficient children and repletion with vitamin A effects a significant increase in hemoglobin and hematocrit. Moreover, Hodges et al. (1) have reported, in experimentally-induced vitamin A deficiency in man, an anemia that did not respond to medicinal iron but did respond to vitamin A

supplementation. Obviously, these observations may have considerable clinical significance in view of the large populations in the world that are deficient in vitamin A and also show signs of anemia. With respect to the data presented, the immature rat in a state of only moderate vitamin A deficiency or the young adult rat that develops vitamin A deficiency more slowly appear to be useful models in investigating various aspects of this phenomenon. By contrast, immature rats that develop vitamin A deficiency quickly may never show evidence of anemia, probably because of a diminution in plasma volume with resultant hemoconcentration.

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