Vitamin E Deficiency in the Monkey.
IV. Further Studies of the Anemia with Emphasis on Bone Marrow Morphology

By F. Stanley Porter, Coy D. Fitch and James S. Dinning

Anemia regularly develops in the rhesus monkey (Macaca mulatta) when it is deprived of vitamin E. The anemia is accompanied by granulocytosis, an increased concentration of bone marrow deoxyribonucleic acid, a reduction in the maximal Cr$^{51}$ survival time of the erythrocytes, a slightly elevated reticulocyte count and a prompt reticulocytosis following vitamin E administration. This combination of observations suggests that the anemia is due to impaired production as well as to decreased life span of the erythrocytes. In the present report, the morphologic abnormalities in the bone marrow from vitamin E-deficient monkeys are described, as well as the results of further studies made in an attempt to elucidate the nature of the hemolytic component of the anemia.

Experimental

Young rhesus monkeys initially weighing between 1.5 and 2.5 Kg. were supplied, ad libitum, with water and vitamin E-deficient purified diets that either contained lard and cod liver oil or no added fat. Two monkeys received the fat-deficient diet and six monkeys received the diet that contained fat. Each monkey was given 0.05 to 0.1 $\mu$g of vitamin $B_12$ per day. One monkey supplied with the fat-deficient diet and two monkeys given the diets containing fat served as controls and received supplements of D, L-alpha-tocopherol, varying from 20 mg. per week initially to 240 mg. per week during the last year of the experiment. The tocopherol was administered either orally as the acetate or intramuscularly as the disodium salt of the phosphate ester.

After being on the experiment for 11 months, the monkeys were exposed to an animal that subsequently died from tuberculosis. All the monkeys were then given isoniazid at a level of 100 mg. per monkey per day. After one month the dose of isoniazid was reduced to 66 mg. (11 to 22 mg. per Kg.) per day and it was maintained at this level throughout the remainder of the experiment. None of the animals in this experiment developed tuberculosis.

At frequent intervals blood was obtained from an ear vein for determination of the hemoglobin concentration, total white blood cell count, reticulocyte count and differential white blood cell count by standard hematologic technics. Since the vitamin E-deficient monkeys exhibited no hematologic abnormalities during the early part of the experiment, the hemoglobin, total white blood cell count and reticulocyte values for these monkeys obtained during the first 15 months are used as part of the control data.

Serum iron by the method of Trinder, platelet counts by the method of Brecher and...
Table 1.—Hematologic Data on Vitamin E-deficient Monkeys

<table>
<thead>
<tr>
<th>Monkey No.</th>
<th>Hemoglobin* Gm./100 ml.</th>
<th>Leukocytes* x 10^9/mm.³</th>
<th>Platelets x 10^9/mm.³</th>
<th>Reticulocytes* %</th>
<th>Serum Iron μg./100 ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.7 ± 0.171 (10)</td>
<td>14.24 ± 0.041 (10)</td>
<td>334 ± 42.5 (10)</td>
<td>0.92 ± 0.02 (10)</td>
<td>178.1 ± 17.4 (10)</td>
</tr>
<tr>
<td>E-deficient</td>
<td>215</td>
<td>6.1</td>
<td>17.5</td>
<td>1,494</td>
<td>0.8 (17.5)</td>
</tr>
<tr>
<td></td>
<td>216</td>
<td>7.2</td>
<td>14.0</td>
<td>310</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>218</td>
<td>8.7</td>
<td>17.5</td>
<td>248</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>221†</td>
<td>6.1</td>
<td>15.5</td>
<td>231</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>222</td>
<td>6.1</td>
<td>10.4</td>
<td>2,310</td>
<td>2.4 (22.2)</td>
</tr>
</tbody>
</table>

*Fifteen to 44 determinations were done on each animal during the control period.  
†± = standard deviation of the mean.  
‡Numbers in parentheses indicate the number of animals used.  
§Reticulocyte response four to five days after either 40 mg. of D, L alpha tocopherol acetate in ethanol orally, or 40 mg. of the disodium salt of D, L alpha tocopherol phosphate in water intraperitoneally.  
This monkey was also on a fat free diet.

Table 2.—Differential Bone Marrow Counts on Vitamin E-deficient Monkeys

<table>
<thead>
<tr>
<th>Monkey No.</th>
<th>Erythroid Precursors</th>
<th>Myelocytes and Precursors</th>
<th>Lymphocytes Cells</th>
<th>Plasma Cells</th>
<th>% Erythroid Precursors Multinucleated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control†</td>
<td>24.8 (8.4-28.8)</td>
<td>48.9 (33.2-81.6)</td>
<td>24.6 (8.4-34.2)</td>
<td>1.14 (0-4.8)</td>
<td>0</td>
</tr>
<tr>
<td>E-deficient</td>
<td>215</td>
<td>46.8</td>
<td>45.6</td>
<td>8.4</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>216</td>
<td>35.6</td>
<td>55.2</td>
<td>9.2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>218</td>
<td>30.0</td>
<td>52.8</td>
<td>6.4</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>221†</td>
<td>26.8</td>
<td>68.0</td>
<td>5.2</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>222</td>
<td>55.0</td>
<td>37.2</td>
<td>5.6</td>
<td>0.8</td>
</tr>
</tbody>
</table>

*The bone marrows were obtained at the same time as the data in table 1.  
†Control values are the average of 14 determinations made on 10 animals. The range is given in parentheses.  
†This monkey was on a fat free diet.

Cronkite,6 glutathione stability of the erythrocytes by the method of Beutler,7 and the osmotic fragility of the erythrocytes after incubation for 24 hours at 37 C. according to Dacie's8 method, were determined at the beginning of the experiment and again when the monkeys exhibited the symptoms of severe vitamin E deficiency. Hemoglobin electrophoresis was done by the method described by Cartwright.9 Bone marrow examinations were done on each monkey from direct smears of material obtained by tibial aspiration.

RESULTS

All the monkeys that were not supplemented with vitamin E developed the typical syndrome of vitamin E deficiency, including muscular dystrophy, creatininuria and anemia after 17 to 28 months. The absence of fat from the diet did not produce hematologic changes in the control monkey nor did it change the character of the anemia in the vitamin E-deficient monkey.

Vitamin E-deficient monkeys exhibited low hemoglobin values with an insignificant increase in reticulocyte counts (table 1). The total white cell counts were unchanged, but there was a significant increase in the absolute number of neutrophils. In two monkeys the platelet counts were quite high, and in the others normal. Serum iron levels were not reduced. The control values obtained in this experiment agree with those reported by others.10-12

The bone marrow from the vitamin E-deficient monkeys exhibited a moderate erythroid hyperplasia and a relative lymphopenia (table 2). Examina-
Fig. 1.—a, b and c show multinucleated erythroid precursors from the bone marrow of a vitamin E-deficient monkey. d. Bone marrow from a monkey during recovery.

tion of these smears showed many multinucleated erythroid precursors. All stages of erythroid development were involved with cells having up to and including four nuclei, but the majority were binucleated (fig. 1). In addition, the nuclear chromatin did not have the typical clumped appearance but
was homogenous and densely staining with a black iridescent quality, the intensity of staining being greater in the periphery of the nucleus. This varied somewhat and a few cells showed some chromatin clumping in the periphery, and some had two or three small bits of densely staining chromatin in the center; however, no nuclei were normal in appearance. Not all of the erythroid cells were multinucleated (the percentage is given in table 2), but all had the above mentioned nuclear changes.

The size of the cells varied directly with the number of nuclei, those with one nucleus being normal in size, those with four being quite large. By visual inspection, the increase in size appeared to be primarily due to an increase in nuclear mass and not to an increase in the amount of cytoplasm, which seemed to be roughly the same for each cell regardless of the number of nuclei present. The cytoplasm itself was normal in appearance, progressing from the dark blue of the pronormoblast to the well hemoglobinized normoblast and the size of the nucleus or nuclei was commensurate with the degree of cytoplasmic maturation. There was no evidence of a maturation arrest and the myeloid cells were morphologically normal as were the megakaryocytes, which were present in adequate numbers. A few mitotic figures were seen but none was multipolar.

Examination of the peripheral blood smears revealed the red cells to be normal in size, shape and degree of hemoglobinization. Some normoblasts were present, however, and a few were binucleated.

The glutathione stability of the red cells was normal and hemoglobin electrophoresis showed a single spot corresponding to type A human hemoglobin.

On recovery of the animals with vitamin E there was a prompt reticulocytosis with an eventual hemoglobin rise. In two animals, repeat examination of the bone marrow at the height of the reticulocyte response showed marked erythroid hyperplasia, but the multinucleated cells and nuclear abnormalities were no longer present (see fig. 1d). The serum iron concentration was determined in one animal during recovery and was found to be 50 μg per cent.

The range of 10 control determinations of red cell osmotic fragility after 24 hours' incubation is shown in figure 2. The vitamin E-deficient monkeys had values well within this range. In two monkeys the red cell fragilities were repeated after vitamin E administration, and demonstrated increased fragility as shown by the curves nos. 216 and 222 in figure 2, the number designation referring to the monkey involved. Monkey No. 222 had been given vitamin E on the 12th and 7th day prior to the repeat determination and monkey No. 216 on the 9th and 3rd day before.

**Discussion**

The anemia observed in vitamin E-deficient monkeys seems to be due primarily to inadequate erythropoiesis, though—as mentioned before—there is a decrease in the Cr51 whole life of the erythrocytes. This view is supported by several observations: there is an inadequate reticulocyte response despite a severe anemia; if the monkeys do not recover with vitamin E the hemoglobin concentration continues to drop until death ensues; and following vitamin E administration there is a prompt and sustained reticulocytosis with
Fig. 2.—Osmotic fragilities of erythrocytes incubated for 24 hours at 37 C. The control values are a composite of a single determination in each of 10 different monkeys. Curves 216 and 222 are from previously vitamin E-deficient monkeys following vitamin E administration.

an eventual rise in hemoglobin concentration to normal, a marked erythroid hyperplasia in the marrow and, in the one instance studied, a decrease in serum iron concentration. These observations are all indicative of inadequate erythropoiesis in the deficient state and restoration to normal with an adequate erythroid response after vitamin E is given.

The presence of multinucleated erythroid precursors in the bone marrow of the deficient monkeys was a surprising and unexpected finding. The multinucleation must occur in the most primitive erythroid cells since it was observed in the earliest recognizable precursors. Wolf and van Hofe,13 in reporting their cases of familial erythroid multinuclearity, reviewed the literature on the subject and found reports of multinucleated erythroid cells present in a variety of severe anemias but most commonly in megaloblastic ones. Bergström and Jacobsson14 have recently described a family in Sweden with a syndrome they named hereditary benign erythroreticulosis, which is characterized by multinucleated erythroid precursors in the bone marrow. They stated that this picture corresponds to that seen in the Di Guglielmo disorder of erythremic myelosis.

Gasser15 has described giant proerythroblasts, some of which are multinucleated, in "acute erythroblastopenia," and Xho Lien-Keng16 has observed these same cells in the marrows of children with kwashiorkor. In the report
of Sundberg et al.\textsuperscript{10} on megaloblastic anemia in the monkey, there are photomicrographs (Plate III) of multinucleated megaloblastic erythroid cells. No reports of the other peculiar nuclear abnormalities observed in this experiment could be found.

That a deficiency of vitamin E results in profound nuclear changes in the erythroid cell, with subsequent inadequate erythropoiesis and decreased Cr\textsuperscript{51} erythrocyte whole life, is evident. Similar phenomena are observed in vitamin B\textsubscript{12} deficiency in humans, and it is tempting to make an analogy since in both instances there are severe nuclear abnormalities which are reversible upon administration of the respective vitamin. However, in the vitamin E-deficient monkeys the myeloid cells and platelets are normal, there are no megaloblastic changes per se in the marrow, and on the peripheral blood smear the erythrocytes appear normochromic and normocytic, though measurements of red cell indices in previous experiments showed the cells to be slightly larger than normal.\textsuperscript{1} It would appear that vitamin E has a considerably different action in nuclear metabolism than vitamin B\textsubscript{12}.

The multinucleated erythroid precursors in the bone marrows of the previously mentioned familial syndromes\textsuperscript{13,14} are similar in appearance to those seen in the vitamin E-deficient monkey. However, in the familial syndromes, anemia, if present, is mild and in one case\textsuperscript{13} there was a tendency to leukopenia and thrombocytopenia. There were also megaloblastic changes in the marrow with some macrocytes in the peripheral blood.

The hematologic changes seen in the vitamin E-deficient monkey have features in common with a variety of previously described syndromes, both acquired and congenital. However, the involvement of only the erythroid cell with abnormalities of both nuclear structure and cytokinesis make this anemia unique. The relationship of vitamin E to these cellular functions is to be the subject of future investigations.

The abnormality in incubated osmotic fragilities of the erythrocytes in the previously deficient monkey following vitamin E administration is an interesting observation and deserves additional study.

**Summary**

Nuclear abnormalities were observed in all the erythroid precursors in the bone marrow of vitamin E-deficient monkeys. Many of these cells were multinucleated. The remainder of the marrow elements appeared normal. Reasons for considering the anemia to be primarily due to inadequate erythropoiesis are given.

Serum iron, glutathione stability of the erythrocytes, hemoglobin electrophoresis, osmotic fragilities and platelet counts were all found to be normal in the vitamin E-deficient monkey.

**Summario in Interlingua**

Anormalitatis nuclear esseva observate in omne le precursos erythroide in le medulla ossee de simias con deficientias de vitamina E. Multes de iste cellulas esseva multinucleate. Le resto del elementos del medulla presentava
un apparentia normal. Es discutite le rationes pro le conception que le anemia es debite primarimente a erythropoiese inadequate.

Ferro seral, stabilitate glutathionic del erythrocytos, electrophorese del hemoglobina, fragilitate osmotic, e numerationes de plachettas, omne istos se monstrava normal in le simia a deficientia de vitamina E.

REFERENCES


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