Biosynthesis of Heme in the Vitamin E-deficient Rat*

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SUMMARY

Vitamin E deficiency in the rat leads to decreased activities of bone marrow δ-aminolevulinic acid synthase and hepatic δ-aminolevulinic acid dehydratase. Studies on the incorporation of radioactivity from glycine-2-¹⁴C and δ-aminolevulinic acid-4-¹⁴C into bone marrow heme show that the defect in this tissue is at the level of the first enzyme, δ-aminolevulinic acid synthase. However, in the liver, the incorporation of δ-aminolevulinic acid-4-¹⁴C into microsomal protoporphyrin in vivo was significantly lower than those in the controls, while no differences were observed when porphobilinogen-¹⁴C was used. Thus, unlike the bone marrow, in the liver, the defect appears to be at the level of the second enzyme δ-aminolevulinic acid dehydratase.

The partial failure to maintain heme synthesis is also reflected in the lowered levels of the heme protein enzymes, catalase, and tryptophan pyrrolase as well as in the levels of microsomal cytochrome b₅ and cytochrome P-450. The nature of the effect is highly specific as indicated by the observation that nonheme enzymes such as tissue ATPase, mitochondrial ATPase, malate, and isocitrate dehydrogenase, microsomal NADPH-cytochrome c reductase, and cytoplasmic glucose-6-P dehydrogenase remain unaltered in vitamin E deficiency. The results suggest that vitamin E functions as a regulator of heme synthesis at one of the rate-limiting steps in the pathway to heme.

Since heme proteins are vital to the maintenance of normal metabolism in the mammalian cell, an aberration in the biogenesis of heme would be expected to give rise to pleomorphic manifestations similar to those described in vitamin E deficiency. The present communication describes experiments indicating the existence of a defect in heme synthesis in the vitamin E-deficient rat.

The results presented here show that vitamin E deficiency leads to a decrease in the ability of the bone marrow to synthesize δ-aminolevulinic acid. In contrast, the locus of the defect in the liver seems to be at the step involving the formation of porphobilinogen.

EXPERIMENTAL PROCEDURE

Materials

Glycine-2-¹⁴C (specific radioactivity, 21.8 mCi per mm) was obtained from Amersham-Searle, Des Plaines, Illinois. δ-Aminolevulinic acid-4-¹⁴C (specific radioactivity, 45 mCi per mm) was obtained from Calatomic, Los Angeles, California. Weanling male rats of the Wistar strain were fed a standard vitamin E-deficient diet for 15 to 18 weeks, unless otherwise stated, before being used for the experiments (5). The corresponding control animals were fed the basal vitamin E-deficient diet supplemented with vitamin E, 200 mg per kg of diet. Rats were killed by exsanguination from the abdominal aorta under mild ether anesthesia.

Vitamin E was dissolved in alcohol and emulsified with a solution of bovine serum albumin to give the appropriate final concentration of the vitamin (7). A similar solution of the vehicle alone was prepared to serve as control. In an alternate method, in studies on microsomal heme synthesis, vitamin E was dissolved in 0.3 ml of ethanol and made up to 10 ml with propylene glycol for intraperitoneal administration.

Porphobilinogen-¹⁴C was prepared enzymatically by the action of rat liver cytoplasmic δ-aminolevulinic acid dehydratase on δ-aminolevulinic acid-4-¹⁴C. Rat liver (15.5 g) was homogenized in 3 volumes of 1.15% KCl and centrifuged at 10,000 X g for 30 min. The supernatant fluid was made up to a volume of 40 ml with 1.15% KCl and distributed equally between two Erlenmeyer flasks. The incubation mixture in each flask consisted of 40 ml of 0.15 M phosphate buffer, pH 6.8; δ-aminolevulinic acid-4-¹⁴C, 45 μCi dissolved in 5 ml of phosphate buffer; nonradioactive δ-aminolevulinic acid, 1.0 μmole, and gluthathione, 200 μmole.

1 The term hemeprotein is used here to represent collectively various molecular forms such as hemoglobin, myoglobin, cytochromes, catalase, and other heme-containing enzymes.

Biologically, vitamin E or α-tocopherol represents the most important member of the class of methyl-substituted tocols. A deficiency of this vitamin is generally characterized by species-specific syndromes such as fetal resorption, defective spermatogenesis, muscular dystrophy, encephalomalacia, exudative diathesis, and degeneration of skeletal and cardiac muscle (1). Recent studies have shown the existence of an anemia associated with a lack of this vitamin in man and in primates. The locus of the metabolic defect has been postulated to reside in the biogenetic sequence leading to heme and hemeproteins (2-5).
μmoles. The mixture was incubated under nitrogen at 37° for 2 hours and the reaction was terminated by the addition of 20 ml of 10% trichloroacetic acid. The precipitated protein was removed by centrifugation, washed once with 5 ml of distilled water, and the combined supernatants were adjusted to pH 5 to 6 with KOH. Porphobilinogen was purified by chromatography on Dowex 2-X8 (8, 9), and eluted from the column with 25 ml of 1 n acetic acid. Its concentration was determined on an aliquot of the eluate with modified Ehrlich’s reagent (8). The rest of the solution was lyophilized to dryness and the residue dissolved in 7 ml of 0.9% sodium chloride solution. This preparation of porphobilinogen had a specific radioactivity of 26.2 mCi per nm.

Methods

Determination of Tissue Tocopherol Levels—Tissue tocopherol levels were determined by gas-liquid chromatographic procedures developed in this laboratory (10-12).

Assay of Hepatic and Bone Marrow δ-Aminolevulinic Acid Synthase Activities—Hepatic δ-aminolevulinic acid synthase was assayed by the procedure of Marver et al. (13). Rat liver tissue was homogenized in 3 volumes of Tris-EDTA buffer, pH 7.4. The incubation mixture containing 2.5 ml of the homogenate, 1 mmole of glycine, 100 μmoles of EDTA, and 750 μmoles of Tris-HCl buffer, pH 7.4, in a total volume of 10 ml was incubated aerobically in a shaker water bath for 1 hour at 37°. The reaction was terminated by the addition of 2.5 ml of 25% trichloroacetic acid, and δ-aminolevulinic acid was determined in the supernatant fluid following its isolation from a Dowex l-X8 column (14). Endogenous δ-aminolevulinic acid was measured simultaneously in a control mixture to which trichloroacetic acid was added at zero time. Results are expressed as millimicromoles of δ-aminolevulinic acid formed per hour per g of tissue.

Femoral bone marrow, aspirated with a hydropemic syringe equipped with a 19-gauge needle, was suspended in 2 ml of Tris-EDTA buffer, pH 7.4. δ-Aminolevulinic acid synthase activity was measured as described earlier (15). Results are expressed as millimicromoles of δ-aminolevulinic acid formed per hour per mg of protein. Protein was measured by the Folin-Ciocalteau procedure.

Assay of Hepatic δ-Aminolevulinic Acid Dehydratase—Hepatic δ-aminolevulinic acid dehydratase was assayed by the procedure of Gibson, Neuberger, and Scott (10). Results are expressed as millimicromoles of porphobilinogen formed per hour per g of tissue.

Incorporation of Radioactivity from Glycine-2-14C and δ-Aminolevulinic Acid-4-14C into Heme by Bone Marrow Cells—The marrow from one femur suspended in Krebs-Ringer phosphate buffer, pH 7.4, was centrifuged for 5 min at 500 x g and reconstituted with the same buffer. The process was repeated twice to obtain a final cell suspension, an appropriate aliquot of which was added to the incubation mixture containing 20 mg of glucose, 20 mg of bovine serum albumin, 1 mg each of penicillin and streptomycin sulfate, and either 10 μCi of glycine-2-14C (specific radioactivity, 2.18 mCi per mm) or 5 μCi of δ-aminolevulinic acid-4-14C (specific radioactivity, 45 mCi per mm) in a total volume of 10 ml. Incubations were carried out aerobically at 37° in 50-ml Erlenmeyer flasks for 2 hours. At the end of the incubation period, the reaction mixture was chilled to 0° and heme was isolated as hemin after the addition of 200 mg of carrier hemoglobin (17). The radioactivity in heme was assayed by liquid scintillation counting.

Assay of Catalase and Tryptophan Pyrrolase Activities in Rat Liver Homogenates—Hepatic catalase (hydrogen peroxide: hydroperoxide oxidoreductase, EC 1.1.1.6) activity was measured as described by Luck (18). One gram of tissue was homogenized with 10 ml of 0.007 m phosphate buffer, pH 7.0, and centrifuged for 10 min at 2000 x g. The pellet was homogenized again in phosphate buffer and recentrifuged. The supernatant fluids from both steps were combined and appropriate dilutions were made for spectrophotometric assay. One unit of catalase activity is defined as that amount of enzyme which liberates 50% of peroxide oxygen from a given concentration of H2O2 in 100 sec at 25°.

Hepatic tryptophan pyrrolase (L-tryptophan: oxygen oxidoreductase, EC 1.13.1.12) was assayed according to a modified procedure of Knox (19) in which the amount of kynurenine formed in 60 min at 37° in the presence of L-tryptophan was measured. Livers were homogenized in 7 volumes of 0.14 M KCl containing 2.5 mM NaOH and 1.0 mM EDTA (20). Duplicate 1-ml portions of this homogenate (equivalent to 125 mg of liver) were incubated at 37° under O2 for 60 min in the presence of 1 ml of 0.2 m phosphate buffer, pH 7.0, 4 μM of hematin (21), and 9 μM of L-tryptophan in a total volume of 4 ml. At the end of the incubation period, the mixture was depurinized with 2 ml of 15% metaphosphoric acid and the amount of kynurenine formed was measured spectrophotometrically at 365 μm (10). The results are expressed as micromoles of kynurenine formed per hour per g of liver, wet weight.

Preparation of Rat Liver Fractions—Vitamin E-deficient rats and the corresponding control animals were killed under ether anesthesia by exsanguination from the abdominal aorta. The livers were rapidly excised, rinsed, and homogenized in a Potter-Elvehjem homogenizer with 10 volumes of cold 0.25 m sucrose containing 1 mM disodium EDTA, pH 7.4. The homogenate was fractionated by differential centrifugation to give the mitochondrial, microsomal, and soluble fractions as described by Baron and Tephy (22).

In studies involving measurement of the incorporation in vivo of labeled precursors into prototrome, microsomal preparations were suspended in a volume of 1.15% KCl equal to that of the original postmitochondrial supernatant and centrifuged at 105,000 x g for 30 min. The purified microsomal pellet which was free of hemoglobin (23), was suspended in 0.1 M phosphate buffer, pH 7.4, and used for the extraction of prototrome.

Enzymatic Assays on Rat Liver Fractions—The mitochondrial preparation as well as an aliquot of the original tissue homogenate was assayed for ATPase (ATP phosphohydrolase, EC 3.6.1.3) activity according to the method of Masoro, Korchak, and Porter (23). Assays of pyridine nucleotide-linked dehydrogenases were based on the extinction coefficient of the difference between reduced and oxidized pyridine nucleotides at 340 μm. The results are expressed as micromoles of NAD or NADP reduced per mg of protein per min. Mitochondria were also used for the measurement of isocitrate dehydrogenase (threo-n,-isocitrate: NADP oxidoreductase, EC 1.1.1.42) (24) and malate dehydrogenase (l-malate:NAD oxidoreductase, EC 1.1.1.37) (25) activities.

The activity of microsomal NADPH-cytochrome c reductase was measured as the rate of increase in absorbance at 550 μm produced by the reduction of cytochrome c (22). The results
are expressed as millimicromoles of cytochrome c reduced per mg of protein per min with the extinction coefficient 19.7 cm$^{-1}$ mmol$^{-1}$ for the difference in absorbance between reduced and oxidized cytochrome c at 550 nm. The activity of glucose-6-P dehydrogenase (p-glucose-6-phosphate:NADP oxidoreductase, EC 1.1.1.49) was measured in the cytoplasmic fraction according to the method of Glock and McLean (26).

Microsomal Cytochrome b$_{5}$ and Cytochrome P-450—Cytochrome b$_{5}$ was determined from the difference spectrum between NADH-reduced and air-saturated microsomes. A value of 163 cm$^{-1}$ mmol$^{-1}$ for the increment in extinction coefficient between 424 and 430 nm was used in computing the concentration of cytochrome b$_{5}$. The results are expressed as millimicromoles of cytochrome b$_{5}$ per mg of protein.

Cytochrome P-450 was determined from the CO difference spectrum of dithionite-reduced microsomes with a molar extinction difference of 91 cm$^{-1}$ mmol$^{-1}$ between 450 and 490 nm (28). The results are expressed as millimicromoles of cytochrome P-450 per mg of protein.

Incorporation in Vivo of Radioactivity from $\delta$-Aminolevulinic Acid-$4^{-14}$C and Porphobilinogen-$14$C into Microsomal Protoheme—Experimental animals fasted for a period of 16 hours received a pulse-dose of either 10 $\mu$Ci of $\delta$-aminolevulinic acid-$4^{-14}$C (specific radioactivity, 45 mCi per mM) or approximately $6 \times 10^{5}$ dpm of porphobilinogen-$14$C (specific radioactivity, 26.2 mCi per mM) prepared enzymatically in this laboratory. The animals were killed exactly 1 hour after the administration of the radioactively labeled precursor and liver microsomes were prepared as described earlier. An aliquot of the microsomal suspension in 0.1 M phosphate buffer, pH 7.4, was centrifuged at 105,000 $\times$ g for 15 min, and the pellet was extracted with 2 to 3 volumes of acetone containing 4% HCl. The precipitated protein was centrifuged and re-extracted with the same solvent. The combined extracts were diluted with an equal volume of water and protoheme was extracted with peroxide-free ether (29).

An appropriate aliquot of the protoheme solution was evaporated in a liquid scintillation vial under a stream of dry nitrogen and dissolved in a mixture of 3 ml of ethylene glycol monobutyl ether and 12 ml of diluted “liquifluor” (Pilot Chemical, Watertown, Massachusetts) for liquid scintillation counting (30).

Radioactivity Measurements—All radioactive fractions were counted in a Packard Tri-Carb automatic liquid scintillation counter (model 3014) equipped with an automatic external standardization device. The counts from each sample were corrected for quenching and expressed as disintegrations per min as described earlier (31).

RESULTS AND DISCUSSION

Hepatic Levels of Vitamin E—In vitamin E-deficient animals the livers have a mean vitamin E concentration of 0.7 $\mu$g per g of tissue compared to about 150 $\mu$g per g of tissue in control animals.

$\delta$-Aminolevulinic Acid Synthase and $\delta$-Aminolevulinic Acid Dehydratase Activities—Since $\delta$-aminolevulinic acid synthase and $\delta$-aminolevulinic acid dehydratase are possibly rate-determining enzymes in the heme biosynthetic pathway, their levels were determined in tissues from vitamin E-deficient animals (Tables I and II). The activity of $\delta$-aminolevulinic acid synthase in bone marrow from deficient animals was only one-half that of the control group (Table I). However, the corresponding hepatic enzyme exhibited only a marginal decrease in its activity, since both control and experimental values were within the normal range reported for the hepatic enzyme (13). Hepatic $\delta$-aminolevulinic acid dehydratase on the other hand was significantly lower than that from control animals (Table II). $\delta$-Aminolevulinic acid dehydratase activities in bone marrow were not reported since existing methods for the assay of this enzyme in hematopoietic tissues were found to be inadequate.

Incorporation of Labeled Precursors into Bone Marrow Heme, in
**To enhance heme synthesis, nonspecifically.**

The ability of deficient bone marrow to incorporate radioactivity from glycine-2-\(^{14}\)C into heme in vitro was approximately one-sixth that of the control bone marrow as seen in Table III. Furthermore, in the same experiment when \(\delta\)-aminolevulinic acid-4-\(^{14}\)C was used as the labeled precursor, no differences were observed in the incorporation of radioactivity into heme in both deficient and control animals. This experiment indicates that in the vitamin E-deficient bone marrow, the defect in heme synthesis is at the step involving the formation of \(\delta\)-aminolevulinic acid.

**Effect of Vitamin E Deficiency on Hepatic Catalase and Tryptophan Pyrroline Activities—**Our observations in earlier experiments showed that the liver in vitamin E deficiency exhibited a lowered level of \(\delta\)-aminolevulinic acid dehydratase activity. It appeared that this enzymatic step could become rate limiting for the synthesis of heme in this tissue and since both catalase and tryptophan pyrroline are hemeoproteins, their activities in the liver would reflect the levels of available heme. As expected, the activities of these two enzymes in vitamin E-deficient livers were significantly lower than those in the corresponding controls (Table VI).

**Effect of Vitamin E Deficiency on Hepatic Microsomal Cytochromes b\(_5\) and P-450—**In vitamin E deficiency, the heme protein components of the microsomal drug-metabolizing enzyme systems were also depressed as shown in Table VII. The levels of

### Table IV

**Effect in vitro of vitamin E on \(\delta\)-aminolevulinic acid synthase, \(\delta\)-aminolevulinic acid dehydratase, and on incorporation of radioactivity from glycine-2-\(^{14}\)C into heme by tissues from vitamin E-deficient rats**

Aqueous emulsions of \(\alpha\)-tocopherol prepared with bovine serum albumin were added to incubation mixtures to give the stated final concentration. An equal volume of vehicle was added to the control tube. Figures within parentheses represent final concentration of vitamin E added to bone marrow cell suspensions only. The amounts of vitamin E added to deficient tissue homogenates were adjusted to approximate concentrations of the vitamin in an equivalent weight of nondeficient tissue. The results are mean of two experiments. Rats were fed a vitamin E-deficient diet for 24 weeks.

| Vitamin E added | \(\delta\)-Aminolevulinic acid synthase | \(\delta\)-Aminolevulinic acid dehydratase | Heme synthesis |
|-----------------|--------------------------------------|----------------------------------------|--------------|
|                 | Liver\(^a\)                          | Bone Marrow                            | Liver\(^b\)  | Protein in bone marrow |
|                 | Per g of liver Specific activity      | Per g of liver Specific activity        | Per g of liver Specific activity | \(\text{dpm} \times 10^{-14}\) mg |
| None            | 14.3                                  | 248.5                                  | 31           | 267                      | 4640                      | 15.2                    |
| 200 (25)        | 10.4                                  | 177.8                                  | 28           | 269                      | 4900                      | 14.8                    |
| 400 (50)        | 12.1                                  | 208.6                                  | 26           | 310                      | 5370                      | 10.1                    |
| 1600 (100)      | 11.7                                  | 107.7                                  | 23           | 308                      | 5300                      | 16.1                    |

*Expressed as millimicromoles of \(\delta\)-aminolevulinic acid produced per hour. Specific activity is expressed per g of protein.

### Table V

**Effect in vivo of vitamin E on \(\delta\)-aminolevulinic acid synthase, \(\delta\)-aminolevulinic acid dehydratase, and on incorporation of radioactivity from glycine-2-\(^{14}\)C into heme by tissues from vitamin E-deficient rats**

Rats were fed a vitamin E-deficient diet for 24 weeks. The vitamin E-supplemented group received orally 100 mg of vitamin E in aqueous emulsion 24 hours prior to being killed. There were four animals in each group, and values represent mean and standard error of mean. In all instances the results are highly significant (\(p\) value < 0.05).

| Group                  | \(\delta\)-Aminolevulinic acid synthase | \(\delta\)-Aminolevulinic acid dehydratase | Heme synthesis |
|------------------------|--------------------------------------|----------------------------------------|--------------|
|                       | Liver\(^a\)                          | Bone marrow                            | Liver\(^b\)  | \(\text{dpm} \times 10^{-14}\) mg protein in bone marrow |
|                       | Per g of liver Specific activity      | Per g of liver Specific activity        | Per g of liver Specific activity | |
| Vitamin E-deficient    | 11.4 ± 1.1                           | 170 ± 15                               | 33.2 ± 1.5   | 191 ± 26                      | 2730 ± 220                | 14.1 ± 1.4               |
| Vitamin E-supplemented | 17.7 ± 1.6                           | 280 ± 16                               | 50.4 ± 2.0   | 444 ± 42                      | 7040 ± 430                | 35.4 ± 3.7               |

*Expressed as millimicromoles of \(\delta\)-aminolevulinic acid produced per hour. Specific activity is expressed per g of protein.

*Expressed as millimicromoles of porphobilinogen produced per hour. Specific activity is expressed per g of protein.

*In addition to the usual constituents, the incubation mixture contained 1.0 ml of isologous serum.*
Effect of vitamin E deficiency on hepatic catalase and tryptophan pyrrolase activities

Enzymatic assays were performed as described under "Experimental Procedure." The experimental animals were maintained on a vitamin E-deficient diet for a period of 18 weeks. Values represent means ± SE of observations from five animals. *p* values < 0.05 were considered significant.

| Groups      | Catalase activity (μM/min) | Tryptophan pyrrolase activity (μM/min) |
|-------------|----------------------------|----------------------------------------|
| Control     | 1721 ± 32                  | 11.6 ± 0.9                             |
| Experimental| 1033 ± 63                  | 4.2 ± 0.7                              |
| *p* values  | <0.05                      | <0.001                                 |

TABLE VII
Concentrations of microsomal cytochrome b5 and cytochrome P-450 in vitamin E deficiency

Animals were maintained on a vitamin E-deficient diet for 17 weeks. They were fasted overnight before being killed by exsanguination from the abdominal aorta. Details of the isolation of microsomes and determination of cytochromes are described under "Experimental Procedure." Results are expressed as means ± SE, from three animals in each group.

| Groups      | Cytochrome b5♂ | Cytochrome P-450♂ |
|-------------|----------------|------------------|
| Control     | 0.72 ± 0.02    | 2.03 ± 0.06      |
| Experimental| 0.52 ± 0.09    | 1.06 ± 0.29      |


α *p* < 0.01.

TABLE VIII
Specific radioactivity of hepatic microsomal protoporphyrin-9C4 pulse-labeled with δ-aminolevulinic acid-4,6-CO or porphobilinogen-7C

In this experiment rats were maintained on a vitamin E-deficient diet for a period of 17 weeks. After 16 hours fasting, each animal received a pulse of either 10 μCi of δ-aminolevulinic acid-4,6-CO, or 6.4 × 10<sup>6</sup> dpm, porphobilinogen-7C, and hepatic microsomes were isolated 1 hour after injection of the isotope. Concentrations of microsomal protoporphyrin were measured spectrophotometrically as its alkaline pyridine-hemochromogen. Details are described under "Experimental Procedure." Results are mean of three animals.

| Groups      | Specific radioactivity of microsomal protoporphyrin-9C4 pulse-labeled with δ-aminolevulinic acid-4,6-CO or porphobilinogen-7C (μCi/mg protein) |
|-------------|----------------------------------------------------------------------------------------------------------------------------------|
| Control     | 9010                                                                                                                            |
| Experimental| 624                                                                  |

cytochromes b5 and P-450 were lower than those in controls by approximately 50%.

*Effect of Vitamin E Deficiency on Nonhemoprotein Enzymes of Liver—*Vitamin E deficiency was without any effect on total hepatic and mitochondrial ATPase activities. Between control and deficient animals, no differences were noted in the activities of mitochondrial malate and isocitrate dehydrogenase, microsomal NADPH-cytochrome c reductase, and cytoplasmic glucose-6-P dehydrogenase, indicating that the defect in the heme-synthesizing system is specific in vitamin E deficiency.

**Incorporation of Labeled Precursors into Hepatic Microsomal Protoporphyrin—**Since δ-aminolevulinic acid synthase activity was not depressed to the same extent as δ-aminolevulinic acid dehydratase in the livers of deficient animals, the results were suggestive of an aberration at the level of the second enzyme. In order to test this hypothesis, microsomal protoporphyrin was pulse-labeled in vivo with either δ-aminolevulinic acid-4,6-CO or porphobilinogen-7C, these being the substrate and product for the enzymatic reaction catalyzed by δ-aminolevulinic acid dehydratase. The results showed that in deficient animals the incorporation of radioactivity from δ-aminolevulinic acid-4,6-CO into protoporphyrin was only about 7% of that in the control animals whereas the difference in the incorporation of label from porphobilinogen-7C into protoporphyrin was not significant (Table VIII).

**Effect of Vitamin E on Specific Radioactivity of Pulse-labeled Hepatic Microsomal Protoporphyrin in Deficient Animals—**When deficient animals were injected with vitamin E, within an hour there was a dramatic rise in the specific radioactivity of hepatic microsomal protoporphyrin, pulse-labeled with δ-aminolevulinic acid-4,6-CO (Table IX). Maximum stimulation of incorporation of radioactivity was obtained at approximately 3 hours following the administration of vitamin E. Between 6 and 9 hours after Vitamin E, the rate of heme synthesis declined to that of controls.

**Locus of Defect in Heme Synthesis—**The results presented in this paper indicate that vitamin E deficiency in the rat results in a partial loss in the ability of cells to synthesize heme. However, the locus of the defect in hematopoietic cells such as the bone marrow appears to be distinctly different from that in non-hematopoietic cells of the adult liver.

In the following simplified outline of the biosynthesis of heme,

\[
\text{Glycine + succinyl CoA} \rightarrow 6\text{-aminolevulinic acid} \rightarrow \text{porphobilinogen} \rightarrow \text{porphyrinogens} \rightarrow \text{protoporphyrin IX} \rightarrow \text{heme}
\]

the first and second steps are catalyzed by the enzymes δ-aminolevulinic acid synthase and δ-aminolevulinic acid dehydratase,
respects. Since deficient bone marrow cells exhibit a low level of δ-aminolevulinic acid synthase activity in parallel with a specific loss in their ability to incorporate radioactivity from glycine-2-14C into heme without any significant change in the pattern of labeling of heme from δ-aminolevulinic acid-4-14C, it is inferred that in hematopoietic cells, vitamin E deficiency results in a defect at the δ-aminolevulinic acid synthase locus. On the other hand, in the deficient liver, δ-aminolevulinic acid dehydratase, the second enzyme of the heme biosynthetic pathway is lowered. This is compatible with the finding that the concomitant decrease in the incorporation of radioactivity into hepatic liver microsomal drug-metabolizing enzymes is strongly favored by our recent studies showing that vitamin E prevents the induction of experimental porphyria (44) and also causes a remission in human porphyria (45), the former through a mechanism specific for vitamin E, involving the regulation of "inducible" hepatic δ-aminolevulinic acid synthase (15, 46).

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