# Blood Tocopherol Values in Normal Human Adults and Incidence of Vitamin E Deficiency. (26631)

PHILIP L. HARRIS, EDWARD G. HARDENBROOK, FREDERICK P. DEAN, ELEANOR R. CUSACK, AND JAMES L. JENSEN*

*Biochemistry Laboratories, Distillation Products Industries, Division of Eastman Kodak Co., Rochester, N. Y.*

Under controlled experimental conditions, the human requirement for Vit. E varies 6-fold or more, depending on other dietary factors—primarily the content of unsaturated fat(1,2). This discovery has stimulated interest in the Vit. E nutriture of populations of normal subjects on practical diets.

For this reason we present in Table I the results of a survey of blood tocopherol levels made in Rochester, N. Y., as part of a comprehensive study of the influence of multivitamin supplementation on blood levels of Vit. A, C, E, and carotene.

The subjects, presumably in good health and receiving adequate diets, were 120 male and 77 female industrial workers, 17 to 64 years of age. At the beginning of the study, before supplementation, the values ranged from 0.36 to 1.80 mg/100 ml. (For tocopherol analysis, the method of Quaife and Harris was used(3) on blood samples drawn in the morning while the subjects were in a fasting state.) In agreement with the mean values found for other populations (Table II), the mean value was 1.05 ± .32 mg tocopherol/100 ml plasma. Thus the present data confirm the generally accepted conclusion that the normal plasma tocopherol level for humans is very close to 1.0 mg/100 ml.

It is important to recognize, as Edwin *et al.*(21) have pointed out in their critical evaluation of analytical procedures used for measuring tocopherols in animal tissues, that most such values represent total reducing materials rather than tocopherol *per se*. Chromenols, reduced ubiquinones, and other antioxidants as well as tocopherols may be present in blood and other animal tissues; these are measured as tocopherol unless such a step as paper chromatography as recommended by Edwin and coworkers is included in the procedure.

TABLE I. Frequency Distribution of Plasma Tocopherol in Normal Adults (Rochester, N. Y.).

| Plasma tocopherol values (mg/100 ml) | No. of persons | Frequency (%) |
|---|---|---|
| .30- .39 | 3 | 2 |
| .40- .49 | 10 | 5 |
| .50- .59 | 6 | 3 |
| .60- .69 | 9 | 4 |
| .70- .79 | 14 | 7 |
| .80- .89 | 13 | 7 |
| .90- .99 | 22 | 11 |
| 1.00-1.09 | 29 | 15 |
| 1.10-1.19 | 34 | 17 |
| 1.20-1.29 | 21 | 11 |
| 1.30-1.39 | 8 | 4 |
| 1.40-1.49 | 8 | 4 |
| 1.50-1.59 | 6 | 3 |
| 1.60-1.69 | 9 | 4 |
| 1.70-1.79 | 4 | 2 |
| 1.80-1.89 | 1 | 1 |
| | 197 | 100 |
| Mean | 1.05 mg ± .32 (S.D.) | |

In future nutritional surveys, measuring some physiological function related to the tocopherol nutriture of the body would be even better than measuring tocopherol *per se*. Such a method—measurement of the susceptibility of red blood cells to hemolysis—is now available.† Increased susceptibility of red blood cells to hemolysis is the earliest indication of Vit. E deficiency, as was discovered first in animals(22) and is now known to apply to human infants(23,24) and adults(1,2).

* Present address: Beebe Laboratories, Inc., St. Paul, Minn.

† The test is easy to do, and the results are unequivocal(25). The red blood cells from a few drops of blood are washed, then incubated with dilute hydrogen peroxide. The amount of hemoglobin released by rupture of red blood cells is measured colorimetrically and expressed as a percentage of complete hemolysis. Persons with adequate vit E nutriture have red blood cells that are completely resistant; those low in tocopherol have red blood cells that show a significant degree of hemolysis—over 20%.

TABLE II. Plasma Tocopherol in Normal Humans.

| No. of subjects | Locale | Mean | S.D. | Reference |
|---|---|---|---|---|
| 122 | Holland | .77 | ± .35 | Engel(4) |
| 14 | New York City | .78 | ± .39 | Hillman and Rosner(5) |
| 74 | Nashville | .89 | ± .20 | Ferguson *et al.*(6) |
| 20 | Italy | .92 | ± .25 | Rindi and Perri(7) |
| 12 | New York City | .96 | ± .33 | Weehsler *et al.*(8) |
| 188 | St. Louis | .98 | ± .30 | Chieffi and Kirk(9) |
| 116 | Hungary | .99 | ± .25 | Kramer(10) |
| 35 | Steilacoom (Wash.) | 1.04 | ± .25 | Van Bruggen and Straumfjord(11) |
| 70 | Birmingham | 1.04 | ± .30 | Harris *et al.*(12) |
| 17 | Rochester | 1.05 | ± .27 | Scrimshaw *et al.*(13) |
| 583 | England | 1.05 | ± .23 | Leitner *et al.*(14) |
| 197 | Rochester | 1.05 | ± .32 | Harris *et al.*, present study |
| 10 | Durham | 1.06 | ± .06 | Darby *et al.*(15) |
| 30 | Philadelphia | 1.08 | ± .29 | Urbach *et al.*(16) |
| 21 | Nashville | 1.09 | ± .17 | Lemley *et al.*(17) |
| 13 | Rochester | 1.20 | ± .22 | Harris and Quaife(18) |
| 30 | Boston | 1.20 | ± .22 | Postel(19) |
| 23 | New Haven | 1.23 | ± .31 | Klatskin(20) |
| 1575 | Weighted mean* = | 1.01 | ± .24 (S.D.†) | |

$$* \text{ Weighted mean} = \frac{\bar{M}_1 n_1 + \bar{M}_2 n_2 + \bar{M}_3 n_3 + \text{etc.}}{N}.$$

$$† \text{ s (stand. dev.)} = \sqrt{\frac{s_1^2 (n-1) + s_2^2 (n-1) + s_3^2 (n-1) + \text{etc.}}{N - K}},$$

where N = No. of individual values (determinations) and K = No. of separate means (studies).

In both children and adults a significant degree of red blood cell hemolysis occurs when serum or plasma tocopherol concentration falls below 0.5 mg/100 ml. Using this relationship, a tentative interpretation with respect to incidence of Vit. E deficiency can be made of the data from the present survey and those previously reported.

The Rochester study shows that 7% of the subjects had values in the deficiency range. Only 3 other surveys gave the distribution in detail; these show that 12% of the Holland subjects, 4% of the Birmingham subjects, and 2% of the British subjects had blood tocopherol values in the deficiency range.

There is not yet enough information available for us to speculate on the diets of the persons having low blood tocopherol values, especially concerning the newly-raised questions: were the $\alpha$-tocopherol intakes (in contrast to total tocopherol) relatively low, or were the unsaturated fat intakes relatively high? Whichever the case, these data show that a significant number of persons had a tocopherol deficiency symptom. Future nutrition surveys, using the blood hemolysis test, will not only supply interesting comparative data on incidence of tocopherol deficiency—they may also show relationships between dietary tocopherol intake and red blood cell hemolysis test values from which tocopherol requirements (minimum daily requirement under a variety of conditions) can be calculated.

*Summary.* The mean tocopherol concentration in 197 factory workers in Rochester, N. Y., was 1.05 ± 0.32 mg/100 ml. About 7% of the subjects had less than 0.50 mg tocopherol/100 ml, the level below which red blood cell hemolysis tests become positive, indicating Vit. E deficiency.

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## Phospholipid Analysis of Cellular Fractions of the Liver Following Administration of Single Dose of Choline.* (26632)

W. E. CORNATZER AND DUANE G. GALLO†
*Guy and Bertha Ireland Research Laboratory, Department of Biochemistry, University of North Dakota School of Medicine, Grand Forks*

Liver mitochondria, nuclei and microsomes are composed of 16-32% of phospholipids on a dry weight basis(1,2). These intracellular units contain enzymes which can oxidize fatty acids and other metabolites(3). Choline-containing phospholipids (lecithins) are probably an integral part of an enzyme system necessary for oxidization of fats and fatty acids since this is only phospholipid, except phosphatidylinositol which is lipotropic. The lipotropic effect of lecithins appears to act on the metabolism of fatty acids in the liver, rather than enhancing their mobilization in the form of plasma phospholipids(4). There is a progressive decrease in concentration in the whole liver of the lecithins and a diminished ability of the liver to synthesize these phospholipids in the dog(5) or rat(6) maintained on a choline-deficient diet. Diets low in protein reduce the level of total liver phospholipids(7,8). Administration of a single dose of choline stimulated lipid phosphorylation as shown by the increase of radioactive uptake of $P^{32}$(9,10,11) which occurs primarily in the lecithin fraction(12), involving mitochondria and nuclei(13). In view of the above findings, experiments were undertaken to determine the phospholipid analysis of various cellular fractions of liver following administration of a single dose of choline in an attempt to ascertain the role of these lipids in mitochondria and nuclei.

*Methods.* Male albino rats of the Wistar strain weighing 100-110 g were maintained on 5% casein-5% fat diet(10) supplemented with 1% guanidoacetic acid (a methyl group acceptor) for 2.5 weeks and divided into 2 groups. To one group various single doses of choline in 1 ml of water (0, 40, 75 and 150 mg) was administered by stomach tube and the animal sacrificed 6 hours later. A single

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† Present address, Mead Johnson, Evansville, Ind.