Serum Tocopherol Measurement: Effect of Unsuspected Impurities in Water[1] (34961)

W. J. UNTEREKER, R. M. H. KATER,[2] AND M. D. DENTON
(Introduced by C. S. Davidson)
Thorndike Memorial Laboratory, Harvard Medical Unit, Boston City Hospital, Boston, Massachusetts 02118

Serum concentrations of tocopherol have been used frequently as a measurement of body tocopherol content in adult man (1–9). We report here simple recovery experiments with *d,l*-alpha-tocopherol added to human serum using two methods (1, 10). A potential source of error which causes interference in one of these methods is described which may account for some of the low values reported.

Materials and Methods. Fresh solutions of *d,l*-alpha-tocopherol (Sigma) served as the standard for measurement of serum tocopherol and recovery experiments. Reagent grade chemicals were used throughout. Thin-layer chromatography plates were purchased ready made (Applied Science).

The screening method used for total serum tocopherol concentration was described by Bieri *et al.* (1). This method incorporates ethanol denaturation, petroleum ether extraction, and spectrophotometric measurement after reaction with alpha, alpha-dipyridyl and ferric chloride [the Emmerie–Engel reaction (12)]. A Coleman junior spectrophotometer (Model 6D) was used. Using this method recovery experiments were performed and tocopherol concentrations were measured in serum collected from 100 presumably healthy adults (11 hospital personnel and 89 blood bank donors).

The second method to be tested was described by Bieri and Prival for the determination of alpha, beta-gamma, and delta tocopherol concentrations in serum (10). This method involves ethanol–pyrogallol denaturation, saponification with concentrated potassium hydroxide and extraction into hexane to which distilled water has been added. The extract is then separated by thin-layer chromatography in benzene, and quenching zones are eluted with ethanol for estimation of the various tocopherols, as in the first method, by the Emmerie-Engel reaction (12). Recovery experiments of standard tocopherol added to serum were performed by this method using the saponification step alone, and saponification followed by thin-layer chromatography.

Results. Serum tocopherol concentrations using the screening method (6) were reproducible and recovery ranged from 95 to 100%. The sera from 100 normal adults contained a mean tocopherol concentration of 1.02 ± SD 0.24 mg/100 ml.

Recoveries of tocopherol standard using the saponification chromatography method (10) were unsatisfactory and varied between 65 and 76% with poor reproducibility. It became apparent that tocopherol recovery was adversely affected by any step which involved the addition of distilled water or the use of aqueous solutions. The distilled water had been prepared by a standard Barnstead still, but had not been deionized. Although qualitative chemical analysis revealed no detectable metallic cations in the distilled water, it was suspected that such ions, capable of catalyzing the oxidation of tocopherol (11), might be present in trace amounts. Recoveries of standard tocopherol were, therefore, repeated using the same method (10),

[1] Supported in part by Public Health Service Grants Nos. AM-09115, AM-5413, and FR-0076 from the National Institutes of Health, Bethesda, Maryland.

[2] Aided by a travel grant from the Postgraduate Committee in Medicine of the University of Sydney, Australia.

TABLE I. Recoveries of *d,l*-alpha-Tocopherol With and Without Addition of EDTA to Distilled Water and Aqueous Solutions.

Serum sample no.	Procedure used	Recovery (%) Without EDTA	Recovery (%) With EDTA
1	Saponification	67	92
2		71	95
3		74	88
4		66	86
5	Saponification and	76	92
6	chromatography	75	95
7		72	95
Mean ± SD		71.6 ± 3.6	91.9 ± 3.4

both with and without the addition of disodium ethylenediaminetetraacetate (EDTA) (0.2%) of distilled water and to aqueous solutions. In both instances standard precautions were used to prevent oxidation of tocopherol by exposure to light, heat, or air. The mean recovery of tocopherol was increased from 72 to 92% by the use of EDTA containing aqueous solutions (Table I).

Discussion. Recovery of *d,l*-alpha-tocopherol from serum (95–100%) using a simple extraction procedure and the Emmerie–Engel reaction proved satisfactory. The normal mean serum tocopherol concentration measured in 100 healthy adults (1.02 ± SD 0.24 mg/100 ml) is in agreement with results obtained by Bieri *et al.* (1) (1.05 ± 0.26 mg/100 ml), Postel (8) (1.20 ± 0.22 mg/100 ml), Overman *et al.* (7) (1.00 ± 0.25 mg/100 ml) and Harris *et al.* (9) (1.05 ± 0.32 mg/100 ml).

Recoveries of standard tocopherol using a method requiring addition of water and aqueous solution were poor (66–76%). However, tocopherol recovery was increased to 86–95% simply by the addition of EDTA to distilled water and aqueous solutions before use, presumably by chelation of trace amounts of metals such as ferrous or cuprous ions known to catalyze oxidation of tocopherols (11). Such an oxidation could explain in part the low concentrations of serum tocopherol reported by Dayton *et al.* (3), Herting *et al.* (5), and Gordon *et al.* (4) in healthy adults. Diplock *et al.* (13) also reported poor tocopherol recoveries in two methods requiring addition of distilled water.

It is recommended that distilled water or aqueous solutions, used in the saponification or extraction of tocopherols prior to assay by spectrophotometric or gas liquid chromatography methods be treated with EDTA to complex trace impurities which may catalyze oxidation of tocopherol during the procedures.

Summary. Recoveries of *d,l*-alpha-tocopherol from serum using a simple screening procedure were found to be satisfactory (95–100%). Poor recoveries (66–76%) using a second method involving aqueous solutions were due to traces of impurity in distilled water and were increased to 86–95% by the addition of disodium ethylenediaminetetraacetate (EDTA). It is recommended that EDTA be added to aqueous solutions used in procedures for extraction or measurement of tocopherol.

We thank Dr. C. S. Davidson for his suggestions and help in preparation of the manuscript.

1. Bieri, J. G., Teets, L., Balavady, B., and Andrews, E. L., Proc. Soc. Exp. Biol. Med. **117**, 131 (1964).

2. Binder, H. J., Herting, D. C., Hurst, V., Finch, S. C., and Spiro, H. M., N. Engl. J. Med. **273**, 1289 (1965).

3. Dayton, S., Hashimoto, S., Rosenblum, D., and Pearce, M. L., J. Lab. Clin. Med. **65**, 739 (1965).

4. Gordon, H. H., and Nitwosky, H. M., Amer. J. Clin. Nutr. **4**, 391 (1956).

5. Herting, D. C., and Drury, E. E., Amer. J. Clin. Nutr. **17**, 351 (1965).

6. Horwitt, M. K., Amer. J. Clin. Nutr. **8**, 451 (1960).

7. Overman, R. S., McNeely, J. M., Todd, M. E., and Wright, I. S., J. Clin. Nutr. **2**, 168 (1954).

8. Postel, S., J. Clin. Invest. **35**, 1345 (1956).

9. Harris, P. L., Hardenbrook, E. G., Dean, F. P., Cusack, E. R., and Jensen, J. L., Proc. Soc. Exp. Biol. Med. **107**, 381 (1961).

10. Beiri, J. G., and Prival, E. L., Proc. Soc. Exp. Biol. Med. **120**, 554 (1965).

11. Lynn, W. S., *in* "Handbook of Physiology, Lipid Peroxidation" (A. E. Renold and G. F. Cahill, eds.), Sec. 5, p. 1349. Williams & Wilkins, Baltimore (1965).

12. Emmerie, A., and Engle, C., Nature (London) **142**, 873 (1938).

13. Diplock, A. T., Green, J., Bunyan, J., and McHale, D., Brit. J. Nutr. **20**, 95 (1966).

Received Apr. 6, 1970. P.S.E.B.M., 1970, Vol. 134.