Rapid Determination of Plasma Tocopherols.*† (19330)

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Since present methods for the chemical determination of blood tocopherols (Vit. E) are inconvenient for routine use, we wish to report that adaptation of the micro method of Quaife and coworkers(1) to a macro scale affords a simple and rapid technic suitable for this purpose. The micro method is excellent where only small amounts of blood are available, but the procedure is time-consuming and requires special equipment and technics. As a macro method, using 1 to 3 ml of serum or plasma, the determination requires only routine clinical laboratory apparatus and technics, and approximately twice as many samples may be run in a single working day. We therefore believe that publication of experimental details will be of assistance to those investigators whose research requires study of Vitamin E in blood or other biological materials.

Method. The procedure follows that of the micro method, involving: (a) Deproteinization of the sample with an equal volume of ethanol; (b) extraction with 5 ml of xylene; (c) estimation of carotene color intensity in a 3 ml aliquot of the xylene extract; (d) and reaction of the extract with 1 ml of ferric chloride (1.2 mg/ml in absolute ethanol) and 3 ml of α, α'-dipyridyl (1.0 mg/ml in *n*-propanol). The extraction is performed in glass-stoppered test tubes shaken mechanically for 10 minutes, and the optical densities are determined in an Evelyn colorimeter, using a 490 mμ filter for carotene and a 515 mμ filter for the ferric chloride-dipyridyl reaction. Reagent blanks and 3 standards are run with each set of determinations, and the carotene correction factor is set up as in the micro method. This factor was found to be 0.39 ($\pm$.03) in our laboratory.

After subtraction of the appropriate reagent blanks from the 490 and 515 mμ densities, the calculation for tocopherol is:

$$\text{mg \% tocopherol} = (D_{515} - .39D_{490}) \times F \times \frac{3}{V}$$

where

$$F = \frac{\text{mg \% D-}\alpha\text{-tocopherol in standard}}{D_{515} \text{ of standard}}$$ (average derived from the 3 standards, 3 ml samples)

and V = Volume of plasma used.

TABLE I. Statistics—Comparison of Methods.

	Macro-method	Micro-method
Avg level (46 subjects)	1.09 mg %	1.12 mg %
Coef. of variation (std. error/mean)	3.4% (2 subjects)	4% (Quaife) (1) (3 subjects)
Mean coef. of variation between both technics (46 subjects)	$= \sqrt{\frac{\left[\frac{\text{Micro-Macro}}{\text{Micro}} \times 100\right]^2}{N(N-1)}} = 2\%$	
Recoveries (added D-α-tocopherol)	99.7%	107% (Quaife) (1)

Forty-six plasma samples were analyzed by the macro and micro methods, each in duplicate, and the results, as shown in Table I, indicate that the two methods give results which are apparently identical. The macro method is now employed in our laboratory for the analysis of blood samples from normal persons and patients with a variety of diseases, and has been found completely suitable for routine use.

Summary. Expansion of the micro method of Quaife for the estimation of blood tocopherols affords a rapid and convenient routine analytical procedure.

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