Note

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Makoto Hayashi, Tsutomu Unemoto, and Komei Miyaki : Improvement on the Colorimetric Determination of Choline with Iodine.

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The most widely used method for colorimetric determination of choline is that reported by Appleton *et al.*[1] In this method, choline is derived to a sparingly soluble choline periodide by the action of iodine reagent and the solution of the periodide in ethylene dichloride is submitted to colorimetry. Many modified methods have been reported,[2~4] one of which is the method devised by Kushner[3] in which choline periodide is extracted, without precipitation, directly with ethylene dichloride. However, this technique necessitated colorimetric determination within two minutes after extraction.

It was found that, in extraction of choline from aqueous solution with ethylene dichloride containing iodine, addition of potassium iodide to the aqueous layer allowed quantitative extraction of choline as its iodide complex. Therefore, optimal concentration of various reagents for determination of choline was examined and quantitative procedure was established, as follows :

Reagents——0.5 *M* acetate buffer (pH 5.0), 0.1 *M* KI reagent, and 0.5% I_2 in ethylene dichloride.

Procedure——To 1.0 cc. of choline sample (containing 0.01~0.15 μmol.), 0.2 cc. of the acetate buffer and 0.1 cc. of KI reagent are added and the mixture is shaken vigorously with 4.0 cc. of ethylene dichloride reagent. This is centrifuged, aqueous layer is discarded, and the ethylene dichloride layer is submitted to colorimetry at 385 mμ, using 4.0 cc. of ethylene dichloride reagent shaken with 1.0 cc. of water, 0.2 cc. of the buffer, and 0.1 cc. of KI reagent, as a blank. Calibration curve is shown in Fig. 1.

The absorption maximum of choline periodide in ethylene dichloride solution is at 365 mμ but the absorption by this procedure appears at 385 mμ (Fig. 2).

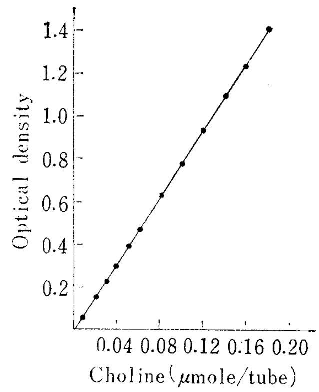

Fig. 1. Calibration Curve of Choline

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1) H.D. Appleton, B.N. LaDu, Jr., B.B. Levy, J.M. Steele, B.V. Brodie : J. Biol. Chem., **205**, 803 (1953).
2) O. Hayaishi, A. Kornberg : J. Biol. Chem., **206**, 647 (1954).
3) D.N. Kushner : Biochim. et Biophys. Acta, **20**, 554 (1956).
4) G. Smits : *Ibid.*, **26**, 424 (1957).

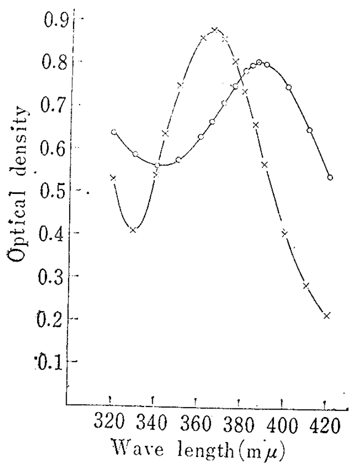

Fig. 2. Absorption Spectra of Choline Periodide
×—× Choline periodide in ethylene dichloride (0.033 μmole per cc.)
○—○ Choline complex against reagent blank by the present method (0.025 μmole per cc.)

This procedure is characteristic in that it enables determination of a micro-amount, to one-tenth of that possible by the past methods[1~4], and the procedure is extremely simple. The procedure, however, is affected to some extent by the presence of trimethylamine and betaines, which did not interfere in the past method of precipitation, unless in fairly high concentration. The effect of these amines was removed by the following treatment.

The effect of the presence of trimethylamine can be removed by pretreatment by Conway's microdiffusion method. Trimethylamine can be determined in a range of 0.02～0.1 μmole/cc. in the same manner as for choline.

In the presence of betaines, the effect can be removed by the use of 0.5 *M* disodium hydrogenphosphate in the place of the acetate buffer, as indicated in Table I. By the means, presence of 50 times of betaines in molar ratio has no effect.

This procedure is also applicable for other tertiary amines and quaternary ammonium compounds (sometimes for secondary amines) and results obtained with amino acids and some pharmaceutics are listed in Table II.

Table I. Effect of Trimethylamine and Betaines

	Amount (μmole)	Smits' method[a)]		Present method		
		Phosphate	0.5*M* AcOH	Phosphate	Acetate	0.5*M* AcOH
		(optical density at 385 mμ)				
Choline	0.10	0.61	0.57	0.72	0.78	0.78
Trimethylamine	0.05	—	—	—	0.41	0.42
	0.10	—	—	0.18	0.80	0.80
	0.15	—	—	—	0.20	1.22
	0.20	0.07	—	0.36	1.55	1.60
	0.30	—	—	0.59	—	—
	0.40	0.60	0.05	0.77	—	—
	0.60	0.95	0.46	—	—	—
	0.80	—	1.26	—	—	—
Betaine	0.10	—	—	—	—	0.31
	0.20	—	—	—	—	0.56
	0.30	—	—	—	—	0.75
	0.40	—	—	—	—	0.90
	1.00	0.01	—	—	—	—
	2.00	—	0.02	0.02	0.11	—
	5.00	0.01	0.02	0.02	0.28	—
Carnitine	0.10	—	—	—	0.11	0.55
	0.20	—	—	—	0.20	0.94
	0.30	—	—	—	—	1.30
	0.40	—	—	—	—	1.65
	0.50	—	—	—	1.39	—
	1.00	0.01	0.01	0.01	0.61	—
	2.00	—	0.38	0.01	—	—
	5.00	0.01	2.00	0.01	—	—

a) For details of the procedure, see reference 4).

Table II. Application of the Present Method to Pharmaceuticals and Biological Amines

Sensitivity as compared with that of choline	Substance
about the same as or more than that of choline	acrinol, procaine, diphenhydramine, atropine, and other tertiary and quaternary ammonium compounds
about 50 per cent	thiamine, ephedrine, tryptamine
10 to 15 per cent (unsuitable for the purpose of quantitative analysis)	tryptophan, ergothioneine, indole-3-acetic acid, hexamine
insensitive	amino acids except tryptophan, B vitamins except thiamine, purines and pyrimidines, creatine, creatinine, histamine, phosphorylcholine, pharmaceuticals (antipyrine, phenacetine, phenobarbital, sulfamine, bromvalerylurea, sulfaguanidine, sulfadiazine, sulfathiazole)

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