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Normal Values and Comparison of Atomic Absorption Spectrophotometric and Fluorometric Methods for Determination of Serum Magnesium

B. S. Tchai, T. S. Park, and Y. G. Suk

College of Medicine Seoul National University, Seoul, Korea.

The diagnostic implications of change in serum magnesium levels have been evident in recent years.[1,2] Serum magnesium level, however, like serum calcium, normally are maintained within very narrow limits so that higly accurate and specific analytical methods are required to detect the relatively small changes which may occur in certain disease states.

The magnesium content of biological fluid has been investigated by many workers using a variety of procedures, including magnesium phosphate precipitation,[3] complexometric titration,[4,5] photometry,[6,7] and emission flame photometry.[8,9,10] Schachter[11] noted the fluorescence of the magnesium compound of 8-hydroxyquinoline in ethanol and used this property for quantitative fluorometric assay.

The fluorometric procedure possesses distinct advantages in that it is specific, accurate, rapid, and employs a very small sample.

Atomic absorption spectroscopy was developed by Walsh[12] as an analytical technic applicable to the determination of many metals. The use of atomic absorption spectroscopy for the determination of magnesium in serum was reported by Willis[13,14] in 1959 and 1960, using direct measurements on serum diluted with an aqueous EDTA solution. Subsequent modifications have involved other direct dilution methods[15-21] or protein precipitation. These published results indicated that atomic absorption spectroscopy would be a simple, rapid, and practical method for the determination of magnesium in biological material, particulary in serum and urine, and that it could be adapted effectvely to the needs of a clinical chemistry laboratories.

As we required an accurate and reproducible method for estimating serum magnesium, it was decided to compare both procedures, fluorometry and atomic absorption spectroscopy. In addition, it is the purpose of this report to complete the normal ranges of magnesium in serum of healthy Korean persons as reference points evaluating pathologic conditions.

Materials and Methods

All glassware is soaked in 50% nitric acid overnight and rinsed well with distilled water and finally with deionized water. All reagent and standards are stored in polyethylene bottles.

Determination of Serum Magnesium by A. A. S.

The atomic absorption spectrometric method was a modfication of the procedure described by Sunderman and Carroll.[22] The instrument

used in this work was Hitach Model 207, connected to a Hitach Model QPD54 recorder. The slot of the burner, which was equipped with disperser for nebulizing samples, is 115x 0.2mm. The power supply to this source is used at 10 ma. and the monochromator is adjusted to measure the absorption of magnesium at a wavelengh of 2852A. An acetylene air flame is used; the acetylene flow rate is 3 l per minute and air is 13L per minute, which provides a blue oxidizing flame. The Hitach Model 207 atomic absorption spectrophotmeter is a single beam instrument with a readout on a meter scale which is linear in absorbance.

Reagents.

1) Magnesium standard, 20mEq/L.

Weigh out 0.2432gm. of metallic magnesium turnings(B. D. H.). Cover with 50ml. of water in a beaker and dissolve by careful addition of conc. HCl dropwise to completely dissolve the magnesium metal. Dilute to 1 liter.

2) Working magnesium standards,

Make a series of working magnesium standards as follow;

ml. stock standard diluted to 100ml.	concentration Mg. (mEq/L).
5.0	1.0
10.0	2.0
15.0	3.0
20.0	4.0

3) Lanthanum-trichloroacetic acid diluent.

Into a 2l volumetric flask are transfered 5.865 gm. of lanthanum oxide (La_2O_3). Concentrated hydrochloric acid (25ml.) is added to the flask and the contents are mixed until a clear solution is obtained. Distilled water (1250 ml.) and 100 Gm. of trichloroacetic acid are added and the conents of the flask are mixed and diluted to the mark.

Preparation of Sample

Into test tubes are transferred 0.05 ml. samples serum, magnesium working stand solutions and water (blank).

5.0ml. of lanthanum-trichloroacetic acid diluent are added to each tube, and the contents are mixed by inversion and allowed to stand for 10 min. The tubes are centrifuged at 2500 r. p. m. for 10 min.

Standard curve

Calibration charts of meter reading (scale divisions) vs. magnesium concentration with ranges of the 1.0 to 4.0mEq/l. curve is given in Fig. 1.

Determination of Serum Magnesium by Fluorometry.

The fluorometric methods was that of Schachter, the original using 8-hydroxyquinone in alcoholic solution. The readings of serum magnesium were made on a Hitach Model 203 fluorescence spectrophotometer, the activating wavelegth being 380nm the fluorescent wavelength 510nm.

Reagents.

1) Acetate buffer, 2.0M, pH6.5

2) Acetate buffer, 2.0M, pH3.5

3) Stock 8-hydroxyquinoline, 5% ethanolic

4) Buffered 8-hydroxyquinoline, pH 3.5 and pH 6.5 Prepare fresh daily mixtures of 30 volumes absolute ethanol, 2 volumes pH 3.5 or 6.5 buffer, and 2 volumes 5% 8-hydroxyquinoline solution.

5) Magnesium Standard solution.

This reagent is prepared as for A. A. S.

Pipette 0.1ml. working standard or test serum into a duplicate series of glass-stoppered test tubes. To one set of tubes add 3.9ml. of pH 3.5 buffered 8-hydroxyquinoline, and to the other and 3.9ml. pH 6.5, buffered 8-hydroxyquinoline. Mix vigorously all tubes for 2 mins. and centrifuge for 15 min. at 200

r. p. m.

The difference in reading between the two tubes is compared with the corresponding difference observed for magnesium standard solution.

Standard curve for measurement of magnesium concentration is given in Fig. 2.

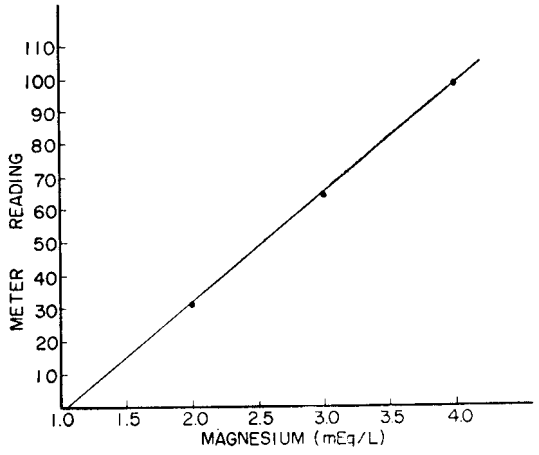

Fig. 1 Calibration Curves for Measurement of Magnesium by Atomic Absorption Spectrophotometer Hitachi Model 207

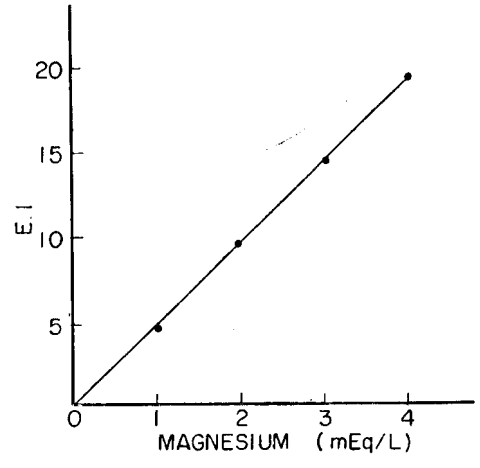

Fig. 2. Standard Curve for Serum Magnesium by Fluorescence Spectrophotometer Hitachi Model 203.

RESUITS AND DISCUSSION

Reproducibility

AAS and fluorometry were tested for reproducibility by analysis of 20 aliquots of pooled normal serum, 20 blanks, and 20 aliquots of a magnesium standard of 2 mEq/L. The results obtained with the fluorometric procedure were as follows:

Coefficient of variation (C. V.) for serum± 2.0%, for standard solution±1.9%. (thus 95%, confidence limits for over all procedure± 4.7%.)

For the AAS method the results obtained were: C. V. for serum±1.8%, C. V. for standard solution±1.4%(and 95% confidence limits ±4.3%).

As can be seen from these results, there is little choice between the two methods on the basis of reproducibility, and both must be termed acceptable.

Normal Values of serum magnesium by A. A. S. and fluorometry.

The values of magnesium in serum of 23 healthy persons, determined with A. A. S. ranged from 1.52–1.90mEq/I. with the mean value 1.72mEq/L. and standard deviation± 0.38 and with fluorometric method according to Schachter, the mean value 1.84(range 1.41–2.22 mEq/L.). and standard deviation±0.45.

Tab. 1 is summerized the results of a number of workers together with those of this study. By the A. A. S. procedure the mean serum magnesium concentration was almost identical with the value reported by Stewart et al and Klein et al.[25] This figure is consistent also with the value of approximately 1.7mEq/L. obtained by diverse methods and cited by Alock[8] et al., but lower than the concentration reported by Wacker at al.[21]

Results of the fluorometric method are a little higher than that of A. A. S. but this is consistent with the serum magnesium concentrations by fluorometry reported by Schachter[26] and Thiers.[27]

Tab. 1. Collected Data on Serum Magnesium Levels in Normal Subjects.

Reference	Method	No.	Mean	Range	Standard	Subjects
Stewart (1963)[20]	AAS	100	1.74	1.30-2.18	Mg. Metal	Blood donors
Wacker (1964)[21]	AAS	10	2.14	1.97-2.31	Mg. Metal	Not specified
Pruden (1965)[23]	AAS	54	1.69	1.43-1.94	$MgSO_4$	Normals
MacDonald (1966)[24]	AAS	30	1.75	1.41-1.85	Mg. Metal	Normals
Tchai (1971)	AAS	23	1.72	1.52-1.90	Mg. Metal	Normals
Schachter (1959)[11]	Fluorometry	33	2.05	1.61-2.49	Mg. oxide	Student nurse
Pruden (1965)[23]	〃	54	2.06	1.62-2.50	$MgSO_4$	Normals
Tchai (1971)	〃	23	1.84	1.41-2.22	Mg. Metal	Normals

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—圖文抄錄—

혈청마구네슘(Mg)정상치 및 원자흡광분광분석법과 형광분석법의 비교

서울대학교 의과대학 임상병리과

채범석 · 박동수 · 석연건

血淸 Mg^{++}의 測定은 Magnesium phosphate precipitation, Complexometric titration, photometry, emission flame photometry, Fluorometry, Atomic absorptions Spectro scopy 等의 여러가지 方法이 있으나 이들中에서 Fluorometry와 Atomic absorption spectro scopy가 가장 정확하고 신속한 方法으로 알려져 있다.

著者들은 이들 Atomic absorption spectro scopy와 Fluoromety 法으로 血淸 Mg^{++}의 韓國人 正常値의 算出과 이들 方法의 재현성에 關한 檢索을 試圖하였다.

Fluorometry의 方法은 8-hydroxyquinone을 사용한 Schachter의 方法으로 Hitachi Model 203 fluorescence spectrophotometer를 사용하였고 Atomic Absorption Spectroscopy는 Sunderman과 Carroll의 方法으로 Hitachi model 207과 Hitach model QPD_{54} recorder를 사용하였다.

재현성검사는 Flucorometry에서 편차항수가 ±4.3%이고 Atomic Absorption Spectro scopy는 ±4.7%이었다. 그러므로 두방법 모두 사용이 가능하다.

正常人 23名의 血淸 Mg^{++}値는 Fluorometry로 1.84±0.45mEq/*l* 이며 측정범위는 1.41~2.22mEq/*l* 이었다. Atomic Absorption Spectro scopy로는 正常値 1.72±0.38mEq/*l* 이며 측정범위는 1.52~1.90mEq/*l* 이었다.