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The determination of metals in blood serum by atomic absorption spectroscopy—II

Magnesium

J. B. WILLIS
Division of Chemical Physics, CSIRO, Chemical Research Laboratories
Melbourne, Australia

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Abstract—The magnesium content of blood serum may be accurately determined by atomic absorption measurements. Analysis can be carried out on as little as 0·05 ml of serum and the only preliminary treatment necessary is dilution of the sample with water containing about 1 per cent of ethylenediaminetetracetic acid or of strontium chloride.

Existing methods for estimation of magnesium

THE lack of detailed knowledge of the functions of magnesium in the body is largely due to the absence of rapid and accurate methods for its routine estimation in biological materials [1,2]. The existing methods are all more or less tedious and require relatively large amounts of sample, so that routine determination of magnesium is not at present practised in many hospital laboratories.

Chemical methods

Apart from the classical procedure in which the magnesium is precipitated as magnesium ammonium phosphate most of the methods which have been proposed for the determination of this metal in serum rely on a complexometric titration with ethylenediaminetetracetic acid and require prior removal of both protein and calcium [3–6]. The quantity of serum required is usually 1–2 ml, though in a recent version of the complexometric method which uses a photoelectric titrator WILKINSON [7] has determined magnesium on as little as 0·1–0·2 ml without prior removal of protein or calcium. HUNTER [8] has described a colorimetric method for measuring the magnesium content of serum after deproteinization, which requires only 0·2 ml of serum but like WILKINSON's method has the disadvantage of determining magnesium indirectly as the difference of two titrations, one giving the calcium content and the other the combined calcium and magnesium content. Errors may also arise from the traces of copper and zinc present in serum.

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Flame photometric methods

The estimation of magnesium by the flame photometer is difficult because of the low intensity of the emission spectrum and of interference by sodium, potassium and phosphate present in the serum.

The method of DAVIS [9], in which the magnesium is first separated by precipitation as its complex with 8-hydroxyquinoline, seems to be accurate though tedious, and requires not less than 1 ml of serum. TELOH [10] has described a self-standardization method in which the interference of other ions is largely overcome by measuring two solutions, one of which is a solution of the serum alone while the other is a similar solution to which a known amount of magnesium has been added. This method requires 2 ml and is claimed to have a standard deviation of 3 per cent in estimations on replicate samples.

It has been shown that magnesium may be estimated on the ash from blood serum and other biological materials by atomic absorption spectroscopy [11] and the present writer has given a brief account of a method for estimating this metal in serum without preliminary ashing [12].

Apparatus

The atomic absorption spectrophotometer described in the preceding paper [13], hereafter referred to as Part I, was used for the present work. The magnesium hollow-cathode tube (resonance line 2852 Å) was run at a current of not more than 10 mA in order to achieve maximum sensitivity in absorption.

Experimental procedure

The materials used were all of analytical quality. Standard magnesium solutions were made up by dilution from a stock solution containing 1000 p.p.m. of magnesium, made by dissolving pure magnesium turnings in the minimum quantity of dilute hydrochloric acid and diluting to volume.

Serum was deproteinized where necessary either by the addition of trichloroacetic acid, as in method (b) of Part I, or by warming with dilute acetic acid [8].

Serum was ashed by heating in a silica or platinum crucible for 12 hr in a muffle furnace at 450–550°C or by repeated treatment with nitric acid at 320°C as described by HUNTER [14]. The ash was dissolved by gentle warming with a few drops of hydrochloric acid.

Solutions of serum for direct measurement were diluted in 5 ml volumetric flasks. The high sensitivity of the atomic absorption method for magnesium allowed of accurate measurements on serum diluted twenty-fold or more.

The standard solutions for calibration were made up to contain the same concentration of suppressing or deproteinizing agent as the serum solutions. Typical calibration curves for magnesium are shown in Fig. 1. They are straight lines.

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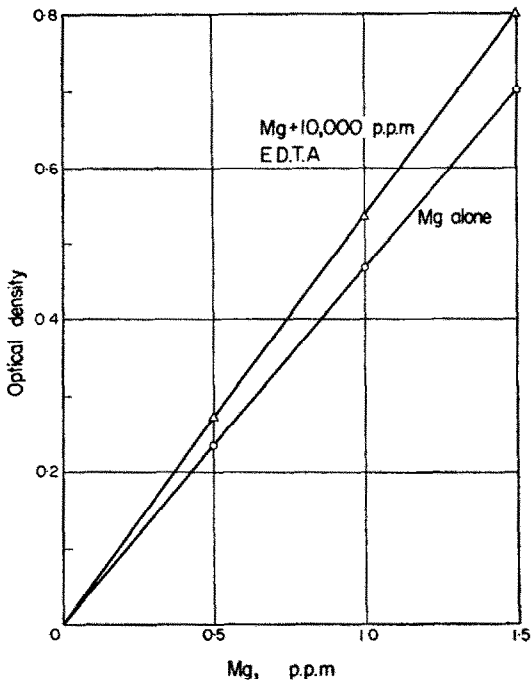

Fig. 1. Typical calibration curves for magnesium in a 10-cm air–acetylene flame.

Interferences in the determination of magnesium

ALLAN [11] has shown that in the air–acetylene flame the absorption of magnesium is unaffected by the presence of sodium, potassium, calcium or phosphate, and the present work confirms this finding. It seemed worthwhile, therefore, to investigate the determination of magnesium in a sample of serum which had been directly diluted with water. Using a lean flame and passing the radiation from the magnesium hollow-cathode through the flame at the height where sensitivity was greatest, the absorptions of different dilutions of serum were measured relative to those of standard solutions containing magnesium alone. The figure obtained for the magnesium content of the serum was independent of the dilution, but the recovery of magnesium added to the solutions was always too high, which indicated that there was probably enhancement of the magnesium absorption by the organic components of the serum.

It is of interest to note that if this solution was also measured after the addition of a known amount of magnesium, i.e. the self-standardization method was used, the value thus obtained for the magnesium content of the serum was identical with that found by use of the two "suppression" methods described below.

Assuming that magnesium, like calcium, combines with protein, it seemed reasonable to suppose that addition of a large quantity of a salt such as calcium or strontium chloride would free the magnesium ions from this combination by a competitive mechanism. It also seemed possible that addition of a large quantity of a substance such as ethylenediaminetetracetic acid (EDTA) would remove magnesium from its combination with protein, and that in such a solution the

magnesium content could be measured by comparison with standards containing the same concentration of EDTA. Table 1 shows that these methods were successful in overcoming the protein interference.

If the absorption was measured near the base of a rich flame, as in the determination of calcium (Part I), it was often found possible to measure serum diluted directly with water. However, since the sensitivity of the absorption method was less when the lower parts of the flame were used, and since careful control of air and acetylene pressures seemed to be necessary, the possibilities of this method were not further investigated.

Results

As with calcium, the lack of a simple analytical method of unquestioned reliability for comparison led to the adoption of the three internal criteria used in Part I.

Table 1. Effect of suppressors on apparent magnesium concentration (Serum 2)

Dilution of serum	Suppressor	Apparent Mg content of serum (mg/100 ml)	Recovery of added Mg (%)
1:12	None	1·99	107–109
1:17	None	2·03	
1:25	None	2·02	
1:50	None	2·01	
1:8	EDTA	1·72	
1:12	10,000 p.p.m.	1·75	
1:17	10,000 p.p.m.	1·74	100–101
1:20	10,000 p.p.m.	1·73	
1:25	10,000 p.p.m.	1·74	
1:50	10,000 p.p.m.	1·75	
1:10	Sr	1·77	99–100
1:20	5000 p.p.m.	1·73	

Table 1 shows that measurements on different dilutions of serum gave magnesium values independent of the degree of dilution for both the Sr and EDTA "suppression" techniques, and, as a further check on the validity of the results obtained, a number of sera were also measured after removal of protein by ashing or coagulation. The results (Table 2) agree with those obtained by the use of the suppression techniques, and such discrepancies as occur can almost certainly be attributed to chemical and manipulative errors associated with the operations of ashing and solution of such small amounts (~ 5 μg) of magnesium. As with calcium, the reproducibility of the results was excellent: the coefficient of variation for a set of replicate serum solutions measured by either of the suppression techniques was 1 per cent or better.

Table 2. Comparison of different methods for estimation of magnesium in serum (mg/100 ml)

Serum no.	Type	Direct dilution EDTA 10,000 p.p.m.	Direct dilution Sr 2500 p.p.m.	Ashed*	Deproteinized*
1	Bovine†	3·56	3·52	3·52	3·53
2	Equine	1·75	1·75	1·80	—
2A	Equine‡	1·35	1·40	—	—
4	Human	2·30	2·27		2·31
7		1·14	—	1·09	1·12
19		2·32	—	—	2·25
20		2·09	—	—	2·22
21		1·94	1·97	—	2·14
22		2·54	—	—	2·44
28		2·59	—	—	2·61
29	Pathol.	2·18	2·16	—	2·27
30	human	1·94	2·00	—	1·99
31		2·25	2·24	—	2·32
32		1·84	1·87	—	—
35		2·07	—	—	2·16
37		2·14	2·10	2·05	—
38		2·10	2·15	2·07	—
39		2·00	1·98	2·00	—

* These solutions contained 2500 p.p.m. Sr, as they were also used for the determination of calcium.
† The magnesium level in this serum ("Chemtrol" freeze-dried serum) is much higher than is normal for bovine specimens.
‡ This serum had stood in the refrigerator for several months, and the precipitate which had formed was centrifuged off before using the supernatant liquid. Some of the magnesium seems to have been lost in the precipitate.

Table 3 shows the result of recovery experiments on solutions to which known quantities of magnesium had been added. It will be noted that the use of EDTA almost always led to recoveries of a little above 100 per cent: the reason for this is not known, but the actual serum magnesium values obtained in the presence of EDTA were no higher than those obtained by the use of strontium chloride.

A few measurements were made with a 10-cm air–coal-gas flame, and it was found that the strontium suppression technique was far more satisfactory than the EDTA technique, since the addition of large concentrations of EDTA lowered the sensitivity markedly and caused the calibration curve to flatten out with increasing concentrations of magnesium. For the few samples measured the values of the magnesium content agreed with those obtained with the air–acetylene flame to within about 3 per cent. It is of interest to note that the absorption sensitivity in the two flames is almost identical, which suggests that the atomization of magnesium is virtually complete even in low-temperature flames.

Conclusions

Estimation of magnesium in blood serum is readily carried out by atomic absorption measurements in the air–acetylene flame on solutions prepared by

Table 3. Results of recovery experiments

Serum no.	Concentration of Mg in serum solution (p.p.m.)	Mg added (p.p.m.)	Total Mg (p.p.m.)	Recovered (p.p.m.)	Recovery (%)
		Direct Dilution with EDTA			
1	1·88	0·50	2·38	2·41	101·3
	1·88	1·00	2·88	2·91	101·0
2	1·40	1·00	2·40	2·42	100·8
2A	0·27	0·40	0·67	0·70	104·5
23	0·59	0·90	1·49	1·49	100·0
	1·53	1·00	2·53	2·48	98·0
31	1·35	0·50	1·85	1·89	102·0
					Av. 100·9
		Direct Dilution with Sr			
29	0·86	0·80	1·66	1·67	100·6
33	1·19	1·00	2·19	2·24	102·3
34	0·66	1·00	1·66	1·64	98·8
					Av. 100·6

direct dilution of the serum with EDTA (10,000 p.p.m.) or strontium chloride (2500 p.p.m. Sr). An air–coal-gas flame is also satisfactory if the strontium addition technique is used. For duplicate measurements 2·5 ml of solution are sufficient, and since the serum can be diluted fifty-fold without much loss in accuracy of measurement the magnesium content of as little as 0·05 ml of serum can be determined.