

THE ESTIMATION OF MAGNESIUM IN SERUM USING TITAN YELLOW

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Recent work in this laboratory in connexion with a study of idiopathic hypercalcaemia in infants has necessitated the estimation of the magnesium content of serum. For this purpose it was decided to use the Kunkel application of the magnesium-titan yellow lake formation (Kunkel, Pearson, and Schweigert, 1947). Despite repeated attempts it was found impossible to produce coloured solutions in which the red lake was sufficiently stable to permit of reliable colorimeter readings.

As a change in technique was found necessary to overcome this difficulty, it was thought that some attempt should be made at the same time to apply a correction for the presence of calcium in the serum. Kolthoff (1927) stated that as calcium intensifies the colour of the magnesium-titan yellow lake, therefore, the Kunkel technique, based on colorimetric comparisons with pure magnesium standard solutions, should result in an over-estimation of magnesium in serum, while it has been claimed by Garner (1946) that "the presence of calcium in concentrations of the order usually found in blood does not affect the colour produced when read at 5,200 Å." On the other hand, it has been shown (Peech and English, 1944; Pieters, Hanssen, and Geurts, 1948) that the addition of 100 parts per million of calcium to the solution of magnesium before formation of lake produces maximal, and therefore uniform, intensification of colour. Irrespective of this conflict of views, it would seem that the addition of calcium to the magnesium standards in the concentration found in serum is sufficient to eliminate any "calcium effect."

The lack of stability of the coloured solutions in our hands was thought to be due to the use of hydroxylamine hydrochloride as a colour stabilizer. The same criticism has been made by other workers (Stross, 1942). Substitution of gum ghatti as a protective colloid produced lake suspensions suit-

able for colorimetric estimation and stable for at least two hours after development (Garner, 1946).

Experimental

Solutions.—The following were used :

0.1% Gum Ghatti.—Powdered gum ghatti, 0.1 g., is suspended in a muslin bag in 100 ml. distilled water for 24 hours. The solution does not deteriorate at room temperature.

0.05% Titan Yellow.—The dye powder, 0.1 g., is dissolved in 200 ml. distilled water.

Stock Standard Magnesium Chloride.—A quantity, 8.458 g. $MgCl_2 \cdot 6H_2O$, is dissolved in distilled water and made up to 1 litre. This solution contains 1,000 μ g. Mg per ml.

Working Standard Magnesium Chloride.—One millilitre stock standard solution is diluted to 200 ml. to give a concentration of 5 μ g. magnesium chloride per ml. Volumes of 1, 2, 3, 4, and 5 ml. made up to 5 ml. in each case and representing 5, 10, 15, 20, and 25 μ g. Mg are used in setting up the standard curve.

Calcium Chloride.—A quantity, 13.88 mg. $CaCl_2$, is dissolved in distilled water and made up to 100 ml., to give a final concentration of 0.05 mg. Ca per ml.

Method.—One millilitre of serum is diluted with 5 ml. distilled water. Proteins are precipitated by the addition of 2 ml. of 10% sodium tungstate and 2 ml. of 0.67 N H_2SO_4 , and the mixture is centrifuged for five minutes at 2,500 r.p.m. To 5 ml. of the supernatant is added 1 ml. distilled water, 1 ml. 0.1% gum ghatti solution, 1 ml. 0.05% titan yellow solution, and 2 ml. 4 N. NaOH. The optical density of the red solution is read using an EEL colorimeter with a 624 filter. A blank using 1 ml. $CaCl_2$ solution containing 0.05 mg. Ca in place of serum is treated similarly to the test. Readings of optical density are converted to magnesium concentrations by reference to a standard curve. This is prepared by carrying out the above colour reaction on 5 ml. samples of solution containing 5, 10, 15, 20, and 25 μ g. Mg, replacing the 1 ml. distilled water added to the protein-free supernatant in the test with 1 ml. $CaCl_2$ solution contain-

ing 0.05 mg. Ca. Distilled water, 5 ml. is used as a standard blank. Instead of setting up the complete standard curve for each set of estimations a single standard may be used: 2.5 ml. of the working Mg standard containing 12.5 μ g. Mg is made to 5 ml. and treated as the test protein-free supernatant, replacing the 1 ml. distilled water with 1 ml. CaCl₂ solution. The standard blank is 5 ml. distilled water.

Calculation.—This is as follows :

$$\begin{aligned} \text{Magnesium in mg./100 ml.} &= \frac{\text{Test reading—blank}}{\text{Standard reading—blank}} \times \text{concentrated standard} \times \frac{100}{0.5} \\ &= \frac{\text{Test reading—blank}}{\text{Standard reading—blank}} \times 0.0125 \times \frac{100}{0.5} \\ &= \frac{T}{S} \times 2.5 \end{aligned}$$

Results

Comparable standard curves were plotted to demonstrate the effects of adding calcium to magnesium solutions. The tubes were set up as shown in Table I.

TABLE I

PROCEDURE FOR DEMONSTRATING EFFECTS OF ADDING CALCIUM TO MAGNESIUM SOLUTIONS ■ ■

Solutions	Tube No.											
	1	2	3	4	5	6	7	8	9	10	11	12
Mg (μ g.) in 5 ml. solution	5	10	15	20	25	—	5	10	15	20	25	—
Ca (mg.) in 1 ml. solution	—	—	—	—	—	—	0.05	0.05	0.05	0.05	0.05	0.05
Water (ml.)	1	1	1	1	1	6	—	—	—	—	—	5
1% gum ghatti (ml.)	1	1	1	1	1	1	1	1	1	1	1	1
0.05% titan yellow (ml.)	1	1	1	1	1	1	1	1	1	1	1	1
4N. NaOH (ml.)	2	2	2	2	2	2	2	2	2	2	2	2

The curves obtained are shown in Fig. 1 and illustrate both the applicability of the Lambert-Beer law within the concentration range likely to be encountered in human sera, and the colour-intensifying effect of added calcium.

Estimations of magnesium in 50 normal sera based on these standard curves gave the following results :

Mg (mg./100 ml. serum) standards containing Ca: mean 2.3, range 1.9–2.7.

Mg (mg./100 ml. serum) standards alone: mean 2.8, range 2.2–3.6.

Failure to allow for the effect of the calcium in serum thus results in an apparent elevation of the magnesium content of about 0.5 mg. per 100 ml.

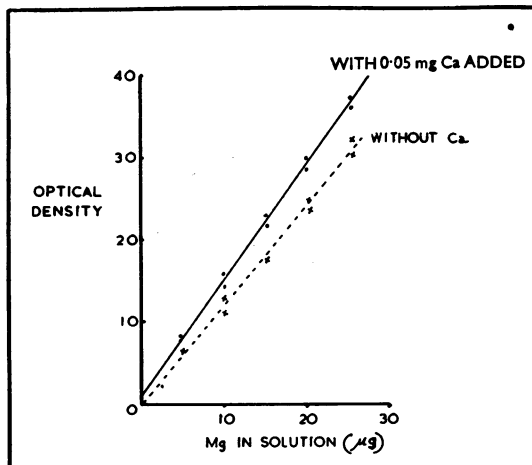


FIG. 1.—Standard curves showing the effect of adding Ca to Mg solutions.

Duplicate estimations on 20 sera gave the following figures :

Mean 2.45 ± 0.24 mg. Mg per 100 ml.

Recovery experiments carried out to assess the efficiency of the technique yielded the results shown in Table II.

TABLE II

RECOVERY OF MG ADDED TO SAMPLES OF SERUM

Test No.	Mg Added (mg. per 100 ml.)	% Recovery
1	0	—
2	0.5	98
3	1.0	95.3
4	2.0	100.0
5	4.0	92.8

Summary

The titan yellow method for estimating serum magnesium is modified by the addition of calcium to the magnesium standard solutions, and by the use of gum ghatti in place of hydroxylamine hydrochloride as a colour stabilizer.

The normal adult magnesium level in serum by this technique is 1.9–2.7 mg. per 100 ml. (mean 2.3).

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