An automated method for serum magnesium estimation

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SYNOPSIS An automated method for magnesium determination in serum is described using conventional AutoAnalyser equipment. The method gives results comparable with those obtained by the flame photometer. The method may prove particularly useful with subnormal serum magnesium levels.

A number of types of method have been used for estimation of magnesium in serum: precipitation as magnesium ammonium phosphate, formation of magnesium hydroxyquinoleate, dye methods, flame-photometry and, more recently, cathode ray polarography (Irving and Watts, 1961) and atomic absorption spectrophotometry (Hurst and McSwiney 1963). Of these only the second and third are at present suitable for use with the AutoAnalyser. Several AutoAnalyser methods using dyes capable of complexing with calcium have been described (Skeggs and Stevens, 1960; Wieme and van Raepenbusch, 1962) and a similar method for magnesium using eriochrome black T is described in the present paper.

Eriochrome black T forms metal complexes with both calcium and magnesium, and the contribution of calcium to the final colour has to be subtracted in order to measure the magnesium complex alone. Hill (1962) has introduced a method for magnesium based on the hydroxyquinoleate method of Schachter (1959) utilizing a continuous flow fluorimeter. He solved the problem of interference from calcium by introducing potassium oxalate into the diluent stream to precipitate calcium as oxalate before dialysis. We have used this method to reduce the calcium correction to a small and fixed proportion of the total deflection.

THE METHOD

The sample of serum is diluted with an acetate buffer at pH 4.0 containing potassium oxalate. At this pH magnesium is released from combination with protein; the potassium oxalate removes calcium which would otherwise contribute to colour development. After dialysis at 37°C. against an aqueous solution of eriochrome

black T, an alkaline buffer (pH 11.6) is added, and after mixing, the absorption is read at 660 mμ. The pH of the mixture in the flow cell is 10.2.

REAGENTS

DILUTING BUFFER Potassium oxalate, 2 g., is dissolved in 1 litre of molar acetate buffer pH 4.0.

STOCK ERIochrome BLACK T SOLUTION Eriochrome black T, 200 mg., is dissolved in 50 ml. absolute methanol. The solution keeps for two weeks at least.

WORKING ERIochrome BLACK T SOLUTION Approximately 4 ml. of stock eriochrome black T solution is diluted in 500 ml. distilled water to which a few drops of Tween 80 are added. This solution should be made up freshly each day.

ALKALINE BUFFER pH 11.6 Dilute 2 ml. 0.880 ammonia and 65 ml. ethanolamine with distilled water to 500 ml.

MAGNESIUM STANDARDS A stock magnesium standard equivalent to 80 mEq./l. is prepared from dry MgSO₄·7H₂O (AR). Working standards equivalent to 0.8, 1.6, 1.4, and 3.2 mEq./l. are prepared by diluting the stock standard.

CALCIUM STANDARDS Stock calcium standard equivalent to 50 mEq./l. is prepared from dry calcium carbonate (Wilkinson, 1957) and working standards prepared by dilution to contain 0.75, 1.5, 3.0, 4.5, 6.0, 7.5, and 9.0 mEq./l.

DESALTED SERUM This is prepared by passing pooled normal serum down a column of ZeoKarb 225 cation exchange resin as described by Wilkinson (1957).

MANIFOLD The manifold shown in Figure 1 was used throughout. The pumping tubes and flow through the dialyser are similar to the arrangement described by Hill (1962).

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FIG. 1. Diagram of manifold for automatic serum magnesium estimation.

NOTES

The exact concentration of eriochrome is not critical. The increase in transmission as the blue colour of the free dye is replaced by the red colour of the dye-metal complex is measured at 660 $m\mu$. Accurate weighing of the eriochrome is not needed once optimal conditions for the method have been determined. Subsequent stock dye solutions may be prepared roughly and added in small increments to 500 ml. of distilled water until the same transmission (usually 25 to 35%) is reached. If the dye solution is much too strong the deflection produced by 1 mEq./l. of magnesium is reduced although the range is increased. Conversely, weak dye solutions are more sensitive to low serum magnesium levels but the dye may be completely converted to the metal complex by magnesium levels in the normal range.

Using the system described the noise level should be less than one half of one small division of the chart. The addition of Tween 80 to the reagents assists the production of a good bubble pattern.

The sampler is set at 40/hr. and a water sample is placed between each serum sample as a significant 'carry-over' occurs if the samples are not separated.

The precipitation of calcium oxalate during dialysis does not block the dialyser or tubing.

RESULTS

THE EFFECT OF PROTEIN BINDING Probably about 35% of the total serum magnesium is bound to protein (Silverman and Gardner, 1954; Prasad, Flink, and McCollister, 1961) although a rather wider range of values has been reported by different authors (Soffer, Dantes, Grossmann, Sobotka, and Jacobs 1939; Gerbrandy, Hellendoorn, De Vries, and Van Leeuwen, 1957). Copeland and Sunderman (1952)

showed that the binding of magnesium by protein obeyed the law of mass action. Albumin and α_2 globulin bind most of the magnesium; α_1 globulin and β globulin may bind some magnesium in pathological states but γ globulin apparently plays no part in magnesium binding (Prasad *et al.*, 1961). In addition 10 to 15% of the bound magnesium (5% or less of the total) may be bound to non-protein substances.

The binding of magnesium by protein decreases as the pH falls, and at the isoelectric point of albumin (pH 5.5) no binding is detectable (Carr and Woods, 1955; Irons and Perkins, 1962). In this work pH 4.0 was selected for dialysis as being well below the isoelectric point of serum albumin and other serum proteins.

Standards were prepared in saline, human albumin at various concentrations up to 4%, and in pooled desalted serum. The calibration curves with saline standards, standards in 4% albumin, and in desalted serum are illustrated in Figure 2. No significant difference was detected, suggesting that under these conditions all the magnesium is available for dialysis. Confirmatory evidence was provided by recovery experiments in which magnesium was added to 19 normal sera (Table II). The average recovery was 97%.

Hill (1962), working with a similar dialysis system, compared ashed and whole sera and concluded that no significant quantity of magnesium remained bound.

THE CALCIUM EFFECT Calcium was added to desalted serum in small increments (Fig. 3) and the

FIG. 2. Calibration curves for magnesium standards in saline, desalted serum, and 4% serum albumin.

magnesium equivalent of the deflection produced is given in Table I. With calcium levels above 3.5 mEq./l. the deflection produced became constant, equivalent to 0.25 mEq./l. of magnesium. At calcium levels above 10 mEq./l. there was some tendency for the calcium effect to increase. Within

TABLE I	
CALCIUM CORRECTION	
mEq./l. Ca	Mg Equivalent (mEq./l.)
0.75	0.16
1.5	0.20
3.0	0.25
4.5	0.25
6.0	0.25
7.5	0.25
9.0	0.25

the range of calcium values likely to be encountered in normal and pathological sera a fixed subtraction of 0.25 mEq./l. should be made from the total to allow for this effect.

Day-to-day variations in this figure were slight but each run should be controlled with a 5 mEq./l. calcium solution in desalted serum. These findings are slightly different from those of Hill (1962) who considered the calcium effect to be no greater than that produced by distilled water.

Figure 4 shows a tracing obtained with a normal serum to which was added calcium in amounts equivalent to 1.25, 2.5, 3.75, and 5.0 mEq./l. The peaks are unchanged by this added calcium.

RECOVERIES

Two types of recovery experiment were performed:

- 1 Stock magnesium standard, 0.1 ml., was added to 10 ml. aliquots of 19 normal sera with an Agla micro-

FIG. 3. Actual AutoAnalyser record of deflections produced by magnesium standards in saline and by calcium and magnesium added to desalted serum.

FIG. 4. An actual AutoAnalyser record of the deflections produced by a normal serum to which calcium up to 5 mEq./l. has been added.

burette in order to raise the magnesium content by 0.8 mEq./l. The magnesium recoveries are given in Table II. The average recovery was 0.775 mEq./l. with a range of 0.70 to 0.85 mEq./l.

2 Automated recoveries were also performed (Hill and Kessler, 1961). In this method a small quantity of magnesium is added to the diluent stream and magnesium determined as before. Although the baseline is shifted, magnesium determination on any given serum, with and

TABLE II

RECOVERY EXPERIMENT ON 19 SERA¹

Serum No.	Magnesium (mEq./l.)	Magnesium (mEq./l.)	Recovery (mEq./l.)
21	1.70	2.55	0.85
22	1.70	2.45	0.75
23	1.60	2.35	0.75
24	1.70	2.55	0.85
25	1.70	2.55	0.85
26	1.45	2.20	0.75
27	1.55	2.30	0.75
28	1.60	2.30	0.70
29	1.35	2.15	0.80
30	1.35	2.15	0.80
31	1.50	2.25	0.75
32	1.45	2.30	0.85
33	1.55	1.25	0.70
34	1.55	1.30	0.75
35	1.50	2.25	0.75
36	1.50	2.35	0.85
37	1.35	2.05	0.70
38	1.55	2.30	0.75
39	1.85	3.60	0.75

Mean 0.775

¹Magnesium was added to each in concentration to raise the serum magnesium by 0.8 mEq./l. The magnesium values have been taken to the nearest 0.05 mEq./l.

without magnesium in the diluent stream, should be the same (Table III). The mean magnesium level of 13 sera was 1.67 mEq./l. After adding magnesium equivalent to 0.5 mEq./l. to the diluent stream the mean was 1.64 mEq./l.

TABLE III

AUTOMATED RECOVERY EXPERIMENT¹

Serum No.	Magnesium (mEq./l.)	Automated Recovery of Magnesium (mEq./l.)
51	1.7	1.75
52	1.8	1.75
53	1.4	1.5
54	1.75	1.80
55	1.8	1.7
56	1.75	1.7
57	1.6	1.5
58	1.7	1.7
61	1.3	1.35
62	1.5	1.5
63	1.8	1.7
64	1.85	1.7
65	1.85	1.8
Mean	1.67	1.64

¹Magnesium was added to the diluent stream in a concentration approximately equivalent to 0.5 mEq./l. of plasma. The magnesium values have been taken to the nearest 0.05 mEq./l.

REPRODUCIBILITY

Five samples of the same serum run successively gave values of 1.68, 1.71, 1.67, 1.67, and 1.74 mEq./l. Four samples of another serum run on different days gave values of 1.79, 1.81, 1.81, and 1.75 mEq./l. This indicates a high degree of reproducibility.

COMPARISON WITH CHEMICAL METHODS

Magnesium estimations were made on 11 sera by this automated method and by the classical method of Briggs (1922). The two methods showed good agreement (Table IV). The mean of the Auto-Analyser method was 0.04 mEq./l. higher than the manual method.

TABLE IV

COMPARISON OF MAGNESIUM DETERMINATIONS ON 11 SERA BY A MANUAL METHOD (BRIGGS, 1922) AND BY THE AUTOANALYSER¹

Serum No.	Manual Method (mEq./l.)	AutoAnalyser (mEq./l.)
1	1.55	1.60
2	1.40	1.45
3	1.50	1.60
4	1.60	1.60
5	1.70	1.70
6	1.90	1.85
7	1.60	1.60
8	1.40	1.50
9	1.80	1.80
10	1.15	1.25
11	1.60	1.70
Mean	1.56	1.60

¹The values obtained have been taken to the nearest 0.05 mEq./l.

FIG. 5. Histogram of magnesium values determined on 69 normal sera.

NORMAL RANGE

The normal range of serum magnesium was determined on 69 sera from blood donors. The results are shown in Figure 5. The mean value obtained was 1.7 mEq./l. with a range of 1.3 to 2.1 mEq./l. The mean agrees well with that obtained by Alcock, MacIntyre, and Radde (1960) with the flame photometer, although the range of the present series is wider.

DISCUSSION

Recent interest in magnesium (Alcock *et al.*, 1960; Booth, Babouris, Hanna, and MacIntyre, 1963) may herald an increase in the number of requests

for estimation of this ion made to the routine clinical laboratory. Although flame photometry has become the best method available, suitable instruments are still few and far between. Many laboratories, however, are being equipped with standard AutoAnalyser equipment for which the present method is designed. Low levels of magnesium appear to be of particular importance (Hanna, 1961) and for this purpose the AutoAnalyser method may prove most useful, as its greatest sensitivity lies in the subnormal range.

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