SERUM MAGNESIUM IN THYROID DISEASE

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Serum magnesium, like serum calcium, is only partly ultrafiltrable (1). Soffer and his associates, in 1939, reported a consistent increase in the non-ultrafiltrable magnesium of the serum in hyperthyroidism (2). In striking contrast, all of the magnesium of the serum was ultrafiltrable in two patients with myxedema. The present work confirms and extends Soffer's observations.

EXPERIMENTAL PROCEDURES

Blood was drawn, with anaerobic precautions, from subjects in the post-absorptive state. Ultrafiltrate was obtained from the serum by an anaerobic technique, by means of the capsule of Lavietes (3), modified in that the diameter of the effective membrane surface was increased to 3 cm., the cellophane membranes were dried immediately before using by pressing between smooth filter papers, and the filtration pressure was reduced to 28 cm. of mercury. More than 3 cc. of ultrafiltrate were obtained from approximately 10 cc. of serum, in 8 to 10 hours.

One cc. aliquots of serum and ultrafiltrate were delivered into 30 cc. porcelain evaporating dishes. To the ultrafiltrate, 2 drops of saturated sucrose solution were added to prevent subsequent loss by crepitation during ashing. With this exception, serum and ultrafiltrate were treated alike. After adding 1 cc. of 4 N H_2SO_4 to each dish, the dishes were placed on a steam bath for at least 2 or 3 hours, to effect charring. Ashing was accomplished in an electric furnace at 500 to 600° C.

The ash was transferred quantitatively to a 6 cc. volumetric flask as follows. One drop of 4 N H_2SO_4 was rubbed into the ash with a short rounded stirring rod, 1 cc. of water was added with further mixing, and transfer was made into the volumetric flask, facilitated by using petrolatum on the lip of the dish. This was followed by 4 washings with 0.5 cc. water. Three drops of 0.1 per cent brom-cresol green were added to the last washing to detect the presence of acid. An additional washing was used if the indicator was not blue or green. If the necks of the volumetric flasks are 6 mm. in inside diameter, the transfer may be made without the use of a funnel.

To the flask was added 1 cc. of a saturated solution of ammonium oxalate, and dilute NH_4OH sufficient to develop a full green color with the brom-cresol green (pH 4.2 to 4.4). The volume was then made to 6 cc. with water and mixed by inversion. After standing at least 3 hours, the contents were transferred to a 15 cc. conical centrifuge tube and centrifuged for 10 minutes. Five cc. of the decanted supernatant fluid were transferred to another 15 cc. conical centrifuge tube. One cc. each of 2 per cent $NH_2H_2PO_4$ and concentrated NH_4OH were then added, and the contents mixed. Precipitation was started by scraping the side of the tube with a sharp-tipped fine stirring rod and mixing thoroughly, and was completed by standing at least 8 hours in the refrigerator. It has been learned subsequently that room temperature is satisfactory for this step.

The precipitate was thrown down by centrifugation for 10 minutes, after which the supernatant fluid was decanted and discarded. The precipitate was washed twice with 8 cc. of dilute NH_4OH (2 cc. of concentrated NH_4OH per 100 cc.), by centrifugation and decantation. Two cc. of the wash solution were first run down the side of the tube and the remaining 6 cc. directed forcefully from a fine-tipped pipette onto the surface of the fluid; this prevented loss of precipitate by floating on the surface. Decantation was done rapidly, leaving behind approximately 0.25 cc. each time. An alternative method of washing, adopted after most of the present data were collected, uses a single washing with 7 per cent NH_4OH. Before the first centrifugation, the volume is made to approximately 12 cc. with the wash solution. The single washing is made by running 2 cc. of the wash solution down the side of the tube, followed by approximately 10 cc. directed forcefully at the surface in a fine stream so as to produce frothing. The supernatant fluid is decanted completely, after which the inner lip of the tube is touched with a towel to remove the adherent drop.

The washed precipitate was dried at 95° C., and determined as phosphate by the method of Benedict and Theis (4). After 1 cc. of the acid molybdate reagent had been added to each tube, complete solution of the precipitate, including any adhering to the sides of the tube, was insured by shaking and rotating. Then 5 cc. of water and 1 cc. of hydroquinone were added, and mixed by inversion. Standard tubes containing 0.02 mgm. phosphorus, as KH_2PO_4, in 5 cc. of water were treated with 1 cc. of the acid molybdate solution, and 1 cc. of hydroquinone sulfite. The tubes were then stoppered lightly with cotton and heated in a boiling water bath for 10 minutes. After cooling and mixing by inversion, the unknowns were compared with the standards by visual colorimetry.

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The calculation is:

$$\frac{24.32}{31.04} \times 0.02 \times \frac{6}{5} \times 100 \times \frac{R}{V} = \text{Mg in milligrams per cent}$$

$$1.88 \frac{R}{V} = \text{Mg in milligrams per cent,}$$

where R is the setting of the standard and V the reading of the unknown.

By this technique, magnesium was recovered from 1 cc. samples of known solutions containing 1.25 to 2.50 mgm. of magnesium as $MgSO_4$ per 100 cc. of solution, with a maximum error of 0.05 mgm. per cent. These solutions contained calcium and phosphorus in amounts comparable to those in serum. All determinations on serum and ultrafiltrate were made in duplicate, the greatest difference between pairs being 0.03 mgm. per cent. As a further check, in the first 6 ultrafiltrations, magnesium was determined in the concentrated residue, as well as in the serum and ultrafiltrate, and the magnesium of ultrafiltrate plus residue was observed to agree almost exactly with that of an equal volume of serum. As an indication of the reproducibility of results, the determinations were repeated under standard conditions in 3 subjects. The constancy was striking (Table I).

TABLE I

Repeated determinations of bound magnesium in 3 subjects

Subject	Serum	Ultrafiltrate	Bound	
	mgm. per cent	*mgm. per cent*	*mgm. per cent*	*per cent of total*
1 A	2.27	1.75	0.52	23
1 B	2.27	1.73	0.54	24
2 A	1.89	1.88	0.01	1
2 B	1.89	1.87	0.02	1
3 A	1.85	1.61	0.24	13
3 B	1.88	1.64	0.24	13 .

The arithmetical difference between the concentrations of magnesium in serum and ultrafiltrate has been taken as bound magnesium, following Soffer's example. Bound magnesium might more properly be calculated by subtracting from serum magnesium, not the magnesium in 100 cc. of ultrafiltrate, but the ionized magnesium of the water of 100 cc. of serum. The latter may be approximated by multiplying the magnesium of ultrafiltrate, which is assumed to be completely ionized, by the Donnan ratio for bivalent ions, and again by the water content of the serum. For normal serum, the Donnan ratio would be approximately $(1.05)^2 = 1.10$, and the water content is approximately 93 per cent. Bound magnesium would, then, become serum magnesium — $(1.10 \times 0.93 \times$ ultrafiltrate magnesium) or serum magnesium — $(1.02 \times$ ultrafiltrate magnesium). Practically, since the water content of serum varies relatively little, and since correction for this almost neutralizes that for the Donnan effect, the distribution of values for bound magnesium is not affected by neglect of these corrections. When they are made in the cases of myxedema, bound magnesium becomes — 0.03 or — 0.04 in all 4 cases of untreated

myxedema, suggesting the possibility that a small amount of the magnesium of ultrafiltrate is unionized.

RESULTS

Bound magnesium has been determined in a group of 14 normal subjects, 9 patients with untreated hyperthyroidism, and 4 patients with untreated myxedema. Blood or serum iodine determination confirmed the clinical diagnoses in these cases, and unmistakable responses to therapy further established them. Total serum magnesium (Figure 1) does not vary significantly in the three groups, but ultrafiltrable magnesium (Figure 2) is distinctly subnormal in hyperthyroidism and
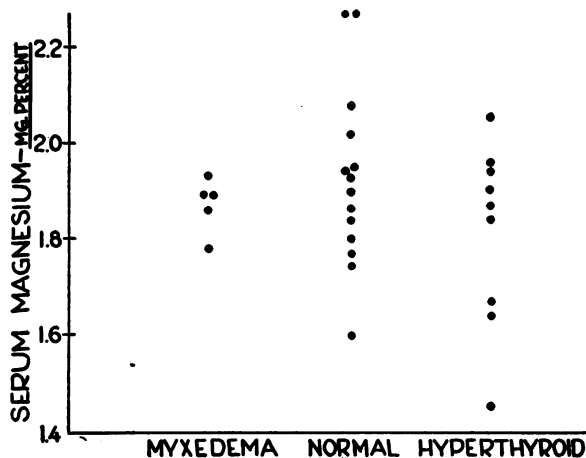

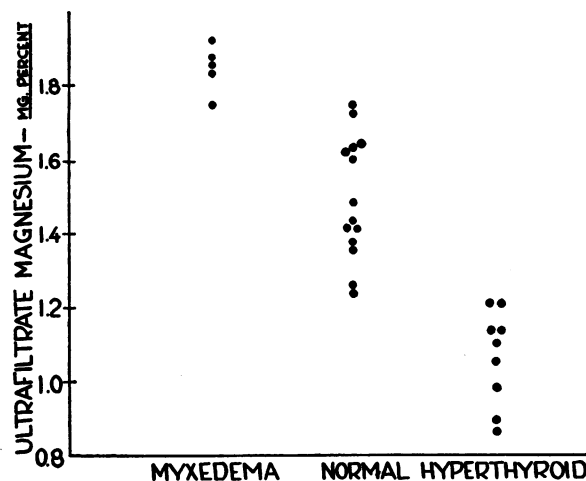FIG. 1. TOTAL SERUM MAGNESIUM IN NORMAL PERSONS AND PATIENTS WITH THYROID DISEASE

FIG. 2. MAGNESIUM OF ULTRAFILTRATE OF SERUM IN NORMAL PERSONS AND PATIENTS WITH THYROID DISEASE

above normal in myxedema, with consequent striking differences in the bound fraction (Figure 3). In myxedema, bound magnesium is regu-
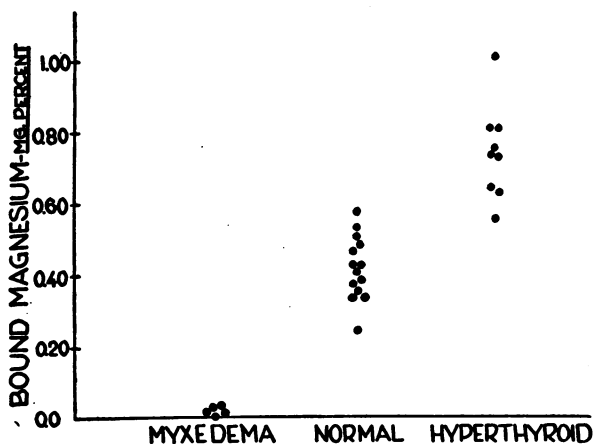

FIG. 3. BOUND MAGNESIUM OF SERUM IN MGM. PER CENT IN NORMAL PERSONS AND PATIENTS WITH THYROID DISEASE

larly absent, and with one exception, and that a minor one, there is no overlapping between the normal and hyperthyroid groups. The differentiation is equally good when bound magnesium is expressed as per cent of the total magnesium (Figure 4). The normal values for bound magnesium fall between 14 and 31 per cent: 12 of the 14 fall within the range 17 to 25 per cent.
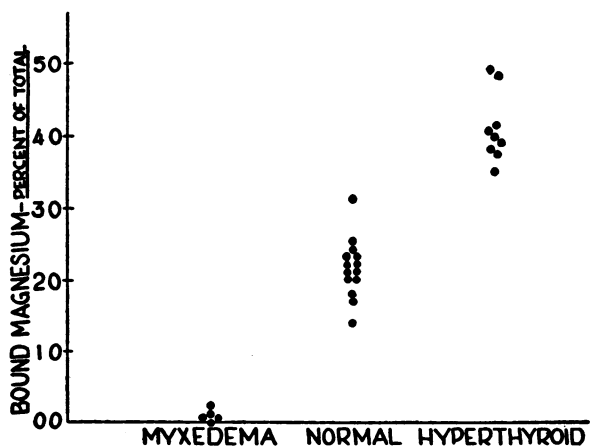

FIG. 4. BOUND MAGNESIUM OF SERUM IN PER CENT OF TOTAL IN NORMAL PERSONS AND PATIENTS WITH THYROID DISEASE

In 5 of 6 patients with hyperthyroidism, studied after the administration of iodine for variable periods, bound magnesium was above the highest normal value (Table II). In the fifth patient,

TABLE II

Hyperthyroidism after treatment with Lugol's solution

Subject	Magnesium			Basal metabolic rate
	Serum	Ultrafiltrate	Bound	
	mgm. per cent	mgm. per cent	per cent	per cent
1	1.69	1.12	34	+44
2	1.94	1.15	41	+47
3	1.74	1.10	37	+22
4	1.89	1.26	33	+26
5 a	2.22	1.41	37	−5
5 b	2.20	1.48	33	+8
6 a	1.26	1.07	15	+42
6 b	1.56	1.12	28	+27

with mild recurrent thyrotoxicosis controlled symptomatically by continuous therapy with Lugol's solution, bound magnesium was elevated on two occasions when the basal metabolic rate was − 5 and + 8 per cent, suggesting that the thyrotoxicosis was still active. When therapy was subsequently discontinued for 3 weeks, the basal metabolic rate rose to + 17, the basal pulse from 70 to 82, and nervousness and palpitation recurred. This is the only patient in the group who was not subjected to thyroidectomy. The first 4 patients, with severe thyrotoxicosis, were first seen by us after courses of iodine therapy of 1 to 6 weeks duration. The sixth patient, with very severe hyperthyroidism, was first studied 3 days after resuming Lugol's solution, after terminating a long previous course about 2 weeks earlier. Bound magnesium was within normal limits at this time, and again a week later; in both instances, however, ultrafiltrable magnesium was subnormal, as was true of the other hyperthyroid subjects. Unfortunately, this patient was not studied before therapy was started; 8 days after operation, total and bound magnesium were both normal.

Bound magnesium was determined in 7 patients with elevated basal metabolic rate, and some of the other stigmata of thyroid excess, such as nervousness, tremor, and tachycardia, but without hyperthyroidism. It fell within normal limits in all (Table III). Blood or serum iodine values were normal in every instance, and at least one course of Lugol's solution was given to each

TABLE III

Hypermetabolism without hyperthyroidism

Subject	Magnesium			Basal metabolic rate
	Serum	Ultrafiltrate	Bound	
	mgm. per cent	*mgm. per cent*	*per cent*	*per cent*
1	1.94	1.56	20	+48
2	1.58	1.22	23	+16
3	2.07	1.71	17	+46
4	2.12	1.65	22	+28
5	1.77	1.21	32	+43
6	1.73	1.38	20	+9
7	1.82	1.38	24	+29
8	1.74	1.49	14	+19

patient without effect on symptoms or metabolic rate, independent evidence that hyperthyroidism was not responsible for the elevated basal metabolism. Two of these patients had marked Parkinsonism, and all of the others had considerable vasomotor and nervous instability.

Three patients with myxedema, adequately controlled by the administration of 2 grains of desiccated thyroid daily, had normal amounts of bound magnesium in their sera. In all of 7 hyperthyroid patients, bound magnesium, which was elevated before thyroidectomy, returned to or below normal limits after operation.

DISCUSSION

Our data give the following indications concerning bound magnesium: (*1*) It varies over a fairly narrow range in normal subjects. (*2*) It is consistently above the normal range in hyperthyroidism, falling towards normal under treatment with iodine, and to normal after thyroidectomy. (*3*) It is entirely lacking in myxedema, returning to normal, however, under treatment with thyroid substance. (*4*) It is normal in patients with abnormal basal metabolism unassociated with thyroid disease. The significance of these observations in relation to diagnosis is self-evident. A recent observation of Soffer (5) that bound magnesium may be normal in some patients with hyperthyroidism, especially when the disease is mild or of long duration, finds no counterpart in our limited experience, although symptoms had been present for more than 2 years in 7 of our cases, and the disease was fairly mild in 3 of these.

Our normal values for bound magnesium are significantly higher and less disperse than those of Soffer. Differences in technique suggest several possible explanations for this discrepancy. For ultrafiltration, Soffer used a pressure of 80 lbs. of nitrogen per square inch. Observations by Flexner (6) indicate that, under such high pressure, ultrafiltrate is not in dialysis equilibrium with the residue, and the discrepancy is in the proper direction to explain the discrepancy between Soffer's data and ours. Watchorn and McCance (1), using low pressure ultrafiltration. observed values comparable to ours. Changes in volume by evaporation or condensation of water during ultrafiltration, possible when gas under high pressure is used, were avoided by our anaerobic technique. Our use of ashes, instead of trichloroacetic acid filtrates, for analysis eliminates possible errors incurred in precipitation of proteins. Our values for total serum magnesium, in distinction to bound magnesium, are approximately 25 per cent lower than Soffer's; since we recovered known solutions quantitatively, this suggests the possibility that their precipitates were impure or incompletely washed.

At present, one can only speculate on the nature of the bound magnesium. Failure to pass through a cellophane membrane indicates that it is incorporated in, or associated with, the colloids of the serum. Unlike calcium, it is not combined with protein in general, being completely absent in myxedema, in which serum proteins tend to be high. Since serum fats, phospholipids, and cholesterol are also elevated in myxedema, it seems improbable that these are responsible for the binding of magnesium. This suggests that it is associated with a specialized protein such as an enzyme or hormone. A rough correlation exists between serum hormonal iodine and bound magnesium in normal subjects and untreated patients, and the increase of each above normal in hyperthyroidism is approximately the same. If a significant correlation between bound magnesium and serum iodine can be established, the magnesium need still not be part of the circulating thyroid hormone, but may rather be associated with the complex in which the hormone functions, possibly an enzyme system. Thus, for example, magnesium is known to serve as an activator for at

least some of the enzymes and coenzymes involved in exchanges of phosphate in intracellular intermediary metabolic processes. It is essential for the activation of adinosinetriphosphate, cocarboxylase, certain dehydrogenases, and kidney phosphatase. Soffer (2) has observed that thyroglobulin does not bind magnesium *in vitro,* and that there is no immediate rise in bound magnesium after the injection of either thyroglobulin or thyroxine into normal dogs. There is a transitory delayed increase after the thyroglobulin, and a prolonged delayed increase after thyroxine, indicating that these substances must undergo modification, or initiate change in other systems, before affecting bound magnesium.

Bound calcium is apparently physiologically inactive, owing its existence only to the chemical affinity of the serum proteins for calcium, the latter being dependent on the resultant of equilibria between the plasma and the gastrointestinal tract, bones, and kidneys. Bound magnesium, in contrast, is probably physiologically active, in a manner quite different from the ultrafiltrable fraction, which, like ultrafiltrable calcium, is presumably essentially completely ionized. The effect of magnesium on excitability of neuromuscular mechanisms is undoubtedly an ionic one. Any explanation of the effect of hyperthyroidism upon serum magnesium, must account not only for the increased binding of magnesium but also for the diminished concentration of ionized magnesium. Ionized magnesium must be the result of the equilibria between serum and gastrointestinal tract, bones, intracellular ionized magnesium, and the kidneys. It is, therefore, difficult to conceive of a mechanism by which increase of bound magnesium results in compensatory decrease in ultrafiltrable magnesium.

Preliminary observations by Dr. Francis P. Vose, in this department, demonstrate an excessive post-absorptive renal excretion of magnesium in hyperthyroidism. Since the concentration of magnesium in ultrafiltrate of serum, and thus presumably in glomerular filtrate, is subnormal in hyperthyroidism, this increased renal excretion must indicate a very large increase and volume of glomerular filtrate, or a diminution of tubular reabsorption of magnesium. The latter is more probably the case since the increases in volume of glomerular filtrate reported in hyperthyroidism (7, 8) are not sufficiently high to explain the increase in urinary magnesium. In any event, the excessive urinary excretion must be responsible, at least in part, for the subnormal concentration of ultrafiltrable magnesium in the serum of hyperthyroid subjects. It cannot explain the extraordinary increase in bound magnesium, which leaves total magnesium normal.

The therapeutic applications of magnesium in thyroid disease are as yet undetermined. One investigator has claimed the regression of symptoms of Graves' disease and a reduction of basal metabolism following injections of magnesium (9). We have not been able to duplicate these results.

SUMMARY AND CONCLUSIONS

A correlation between thyroid function and the state of the serum magnesium has been observed, confirming previous work.

Refinements of technique are described.

In 14 normal subjects, the non-ultrafiltrable, or bound, fraction of the serum magnesium was 17 to 31 per cent of the total. In each of 9 proved untreated cases of hyperthyroidism, bound magnesium exceeded these values, and ultrafiltrable magnesium was subnormal. In 4 patients with myxedema, all of the magnesium of the serum was ultrafiltrable. After therapy of thyroid dysfunction, bound and ultrafiltrable magnesium return to normal. In 8 patients with hypermetabolism without hyperthyroidism, bound magnesium was normal.

The possible relation of bound magnesium to the circulating thyroid hormone or to enzyme systems is discussed.

Attention is directed to the anomalous reciprocal changes of bound and free magnesium.

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