48. The Ultrafilterable Serum Magnesium in Hyperthyroidism

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Recently, Soffer, Dantes, Grossman, Sobotka & Jacobs [1939] have reported that in hyperthyroidism the percentage of the total serum magnesium which is not ultrafilterable is significantly higher than in the serum of normal persons or of persons cured of hyperthyroidism by thyroidectomy. In a group of 14 normal subjects they found that 3-22% of the total serum Mg was not ultrafilterable and was thus apparently bound to protein. In a group of 31 cases of hyperthyroidism, however, the percentage thus bound varied from 21.5 to 61.6% although the total serum Mg was unchanged. After treatment by subtotal thyroidectomy the percentage of bound Mg fell again to normal values, varying in 12 cases from zero to 20.1%.

These results appeared to indicate an association between the thyroid gland and Mg metabolism which had hitherto received little attention. It seemed furthermore, that they might be correlated with the symptomatology of hyperthyroidism, for the rise in bound serum Mg in this condition was associated with a drop in the concentration of ultrafilterable, and so probably of ionizable Mg. In experimental animals a low serum Mg produced by Mg deficiency is frequently associated with hyperirritability of the nervous system and vasomotor instability, and both of these conditions are common in hyperthyroidism. The results claimed by this group of workers were therefore of considerable interest and merited careful confirmation. We have attempted such confirmation, but our results, in contrast to those of Soffer and his co-workers, show no difference in the percentages of bound and ultrafilterable Mg between the sera of cases of hyperthyroidism and the sera of normal persons. Since our results were obtained Soffer, Cohn, Grossman, Jacobs & Sobotka [1941] have extended the earlier observations to a rather larger series though with less clear-cut results. Of 50 patients with hyperthyroidism 15 were found to have a bound serum Mg within the normal range, that is, below 25%. The remaining 35 cases showed a bound serum Mg higher than was found in normals. No correlation was demonstrable between the bound serum Mg and the basal metabolic rate. Laviates & Dine [1941] also, in a preliminary note, claim to have confirmed the main findings of Soffer *et al.* They suggest that Mg may be an integral part of the circulating thyroid hormone complex.

In view of the interesting bearings of these suggestions we present here the details of our own negative findings since they indicate that an association between bound serum Mg and human hyperthyroidism cannot be regarded as fully established.

METHODS

Serum was obtained by a uniform procedure, allowing venous blood to clot for 1 hr. at 37° and for a further hour at room temperature. Calcium was precipitated from a trichloroacetic acid filtrate by oxalate in buffered solution at pH 5.0 and Mg was then precipitated from a known fraction of the supernatant fluid as the Mg ammonium phosphate, the technique to this point being essentially that described by Peters & Van Slyke [1932]. The phosphate content of the washed Mg precipitate was determined colorimetrically using molybdate and stannous chloride according to the modified technique of Berenblum & Chain [1938] in which the blue colour is developed in isobutyl alcohol solution. This modification was used for the final colorimetry because a more linear

correspondence was obtained between colour and phosphate concentration than when the colour was allowed to develop in aqueous solution. Using this technique Mg could be determined in known aqueous solutions with an error not exceeding 3% when the original concentration corresponded to that of a protein-free serum filtrate. Known quantities of Mg added to serum could also be recovered with an error not exceeding 3%.

Ultrafiltration was done on a part of each sample of serum, using a '300' cellophane membrane and an approximately constant air pressure of about $2\frac{1}{2}$ atmospheres. The Mg contents of known aqueous solutions were unaltered by this process and no change in the Mg contents of serum ultrafiltrates took place when they were passed a second time through a cellophane membrane. Mg was determined in the ultrafiltrates by the same technique as was used for the trichloroacetic acid filtrates of the original serum. All ultrafiltrates were shown to be protein-free before analysis. Mg estimations in both serum and ultrafiltrates were done in duplicate and agreement was within 4%.

RESULTS

A series of 17 normal subjects has been examined. It consisted of 13 women and 4 men all of whom had been admitted to hospital for appendicectomy or hernia repair and from whom blood was taken a few days before discharge from hospital and never less than 10 days after operation. In all cases convalescence had been quite uneventful. With three exceptions their ages were between 15 and 28 years. Total serum Mg was found to be relatively constant, the extremes being 1.75 and 2.45 mg./100 ml. and the mean 1.99 mg./100 ml. Ultrafilterable Mg was more variable. In 13 of the 17 cases it lay between 1.07 and 1.60 mg./100 ml. but of the remaining four, one was 2.10 and the other three 0.5, 0.87 and 0.94 mg./100 ml. The mean value for the whole group was 1.25 mg./100 ml. The bound or non-diffusible Mg, obtained by difference, varied from 0.23 to 1.27 mg./100 ml. with a mean of 0.74. Expressed as a percentage of the total serum Mg, the non-diffusible fraction represented 14.2-56% with a mean of 37% of the total.

In hyperthyroidism comparable figures have been obtained in a series of 24 cases composed of 20 women and 4 men. The basal metabolic rate varied from +75 to +16%, but in all except 4 cases exceeded +23%. The mean figure was +39.4%. Blood samples were all taken in this group before any Lugol's iodine had been given. Total serum Mg varied from 1.35 to 2.50 with a mean of 1.88 mg./100 ml. Ultrafilterable Mg ranged from 0.66 to 1.66 with a mean of 1.22 mg./100 ml. and bound Mg, varying from 0.21 to 1.00 mg./100 ml., represented 15-57% of the total with a mean of 35%. In 13 of these cases fresh determinations of the bound Mg were made after 12-14 days on Lugol's iodine and before thyroidectomy. No consistent change was observed; in some the bound fraction rose, in others it fell. The range was wide, from 8.7 to 57% of the total, but the mean figure was 37.3%.

Table 1. *Results of the present investigation*

Type of case	No. of cases	Mean serum Mg, mg./100 ml.			Percentage of total Mg bound
		Total	Filterable	Bound	
Normals	17	1.99	1.25	0.74	37.0
Hyperthyroidism	24	1.88	1.22	0.67	35.0
Hyperthyroidism after iodine	13	1.83	1.15	0.68	37.3
Hyperthyroidism after operation	18	2.03	1.32	0.71	35.8
Anxiety states	4	2.12	1.41	0.71	33.3

Results of Soffer et al. [1939] for comparison

Normals	14	2.52	2.15	0.37	14.5
Hyperthyroidism	31	2.44	1.58	0.86	36.0
Hyperthyroidism after iodine	13	2.54	1.92	0.62	24.0
Hyperthyroidism after operation	12	2.61	2.24	0.37	13.7

In 18 of the original 24 cases further blood was obtained for analysis 10 days or more after the operation of subtotal thyroidectomy. The post-operative basal metabolism was determined in 12 of these cases and gave a mean value for the group of +3%. Bound Mg again varied within wide limits (10–60%) but the mean figure was 35.8%. In individual cases the effect on the bound Mg in the serum of relief of the hyperthyroidism was quite inconstant and unpredictable. In one there was a rise from 20 to 60, in another a fall from 50 to 30%, and in yet other cases there was no appreciable change.

In order that our results may be compared with those of Soffer *et al.*, both are summarized in Table 1.

DISCUSSION

The results in our own series of cases are in marked contrast to those of Soffer *et al.* Although variations occur in the Mg values for individual cases under each heading, yet when averages are taken there is essential uniformity in all groups. The differences in free, bound and total serum Mg between normal persons and those suffering from hyperthyroidism are very small and scarcely exceed the experimental error. We do not regard them as indicating any significant deviation from the normal in hyperthyroidism. After treatment with iodine, and again after thyroidectomy, the figures are also essentially the same as those found in normal subjects. The figures of Soffer *et al.* differ from our own in two respects. First their total serum Mg values average about 0.5 mg./100 ml. higher than our own for all groups. Since these differences occur in all groups it seems possible that they are due to differences of technique. Our own averages of 1.8 to 2.1 mg./100 ml. correspond well with figures obtained by one of us in a previous investigation [Cope, 1935–6]. It is possible that differences in the manner of separation of the serum account for Soffer's higher values. In the partition of ultrafilterable and bound Mg their figures agree with our own for untreated hyperthyroidism; the discrepancy lies mainly in the figures for normal and post-thyroidectomy cases. In these groups Soffer *et al.* found much lower proportions of bound Mg than we did. The reasons for these differences in result are not clear. It does not seem possible to explain them on a basis of differences in method of estimation of the Mg concentration. In preparing our ultrafiltrates we used a thinner grade of cellophane (300) than did Soffer *et al.*, but our ultrafiltrates were always protein-free and the Mg fraction retained was not altered by a second ultrafiltration. It is difficult to believe that our thinner membrane was retaining more bound Mg than their thicker one but we have nevertheless investigated the point. Four batches of serum were obtained from normal subjects and two separate ultrafiltrates were made from each, one with '300' and the other with '600' cellophane, the latter being the thickness used by Soffer. The Mg concentrations in both ultrafiltrates were found to be practically the same. The differences were within experimental error and were quite inadequate to explain our failure to obtain results similar to those of Soffer *et al.*

Sample	Total serum mg.	Cellophane '300'		Cellophane '600'	
		Free	Bound	Free	Bound
1	2.25	1.55	0.70 (31%)	1.51	0.74 (32.9%)
2	1.76	1.35	0.41 (23%)	1.36	0.40 (22.7%)
3	2.08	1.40	0.68 (32.8%)	1.38	0.70 (33.8%)
4	1.92	1.05	0.87 (44.5%)	1.15	0.77 (40.0%)
Mean	2.00	1.34	0.66 (32.8%)	1.35	0.65 (32.4%)

From our own observations we must conclude therefore that there is no deviation from the normal in the amount of Mg bound to protein in the serum of persons with hyperthyroidism, and further, that this fraction is not appreciably influenced by iodine administration or by thyroidectomy. This being so it does not seem possible to attribute the hyperirritability of the nervous system in this disease to a deficiency of ionizable

Mg in the blood serum. Nor do our results support the suggestion that the bound Mg may be part of the thyroid hormone complex, for no relation has been found between the severity of the hyperthyroidism as judged by the basal metabolic rate or the sleeping pulse rate, and the amount of bound Mg in the blood serum.

SUMMARY

An investigation has been made of a recent claim that in hyperthyroidism the percentage of the total serum Mg which does not pass through an ultrafilter and is apparently bound to protein, is significantly higher than in normal persons or in persons cured of hyperthyroidism by operation.

No confirmation of this claim has been obtained, the ultrafilterable and non-ultrafilterable fractions of the serum Mg having been found essentially the same in hyperthyroidism, either before or after treatment, as in normal persons.

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