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Calcium-Protein Relation in Hyperproteinemia: Total and Diffusible Serum Calcium in Lymphogranuloma Inguinale and Myeloma.

ALEXANDER B. GUTMAN AND ETHEL BENEDICT GUTMAN.

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City.

A direct proportionality between calcium and protein concentrations has been demonstrated in sera of less than normal protein content where there was no disturbance of calcium metabolism.[1] This relation could be expressed by an empirical regression equation:[2, 3]

I. Total Ca $= m \cdot$ total protein $+ b$ (where m and b are constants).

It follows from equation I—and it is generally inferred, provided hyperphosphatemia is not present—that elevated calcium values are to be expected in sera of *high* protein content. This plausible inference would seem to be justified by the established fact that a significant proportion, approximately half, of the total calcium in normal serum is bound by protein. The inference appears to be supported by the co-existence of hyperproteinemia and hypercalcemia in cases of multiple myeloma.

However, in lymphogranuloma inguinale, in which the serum protein content may exceed 10%, hypercalcemia is not observed[4, 5] (Table I); nor do we find hypercalcemia accompanying hyperproteinemia encountered occasionally in hepatic cirrhosis, lymphosarcoma, tuberculosis, etc.[5] In multiple myeloma, moreover, the association of hypercalcemia with hyperproteinemia is erratic.[6, 7] Of 57 published cases of multiple myeloma in which both protein and calcium were determined (including 14 of our own), hyperproteinemia occurred in 35; of which number, hypercalcemia was present in 23. In 18 cases of multiple myeloma, hypercalcemia was associated with

1 Salvesen, H. A., and Linder, G. C., *J. Biol. Chem.*, 1923, **58**, 617.

2 Hastings, A. B., Murray, C. D., and Sendroy, J., Jr., *J. Biol. Chem.*, 1927, **71**, 723.

3 McLean, F. C., and Hastings, A. B., *J. Biol. Chem.*, 1935, **108**, 285.

4 Williams, R. D., and Gutman, A. B., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 91.

5 Gutman, A. B., Gutman, E. B., Jillson, R., and Williams, R. D., *J. Clin. Invest.*, 1936, **15**, 475.

6 Robbins, C. L., and Kydd, D. M., *J. Clin. Invest.*, 1935, **14**, 220.

7 Cantarow, A., *Am. J. Med. Sci.*, 1935, **189**, 425.

normal or low serum protein levels. In several instances, the serum calcium remained unchanged or fell as the serum protein content rose; or falling protein levels were accompanied by unchanged or higher calcium levels.

Contrary to prevailing opinion, the direct proportionality between calcium and total protein in sera of low or normal protein content does not obtain in sera with increased protein content (Fig. 1).

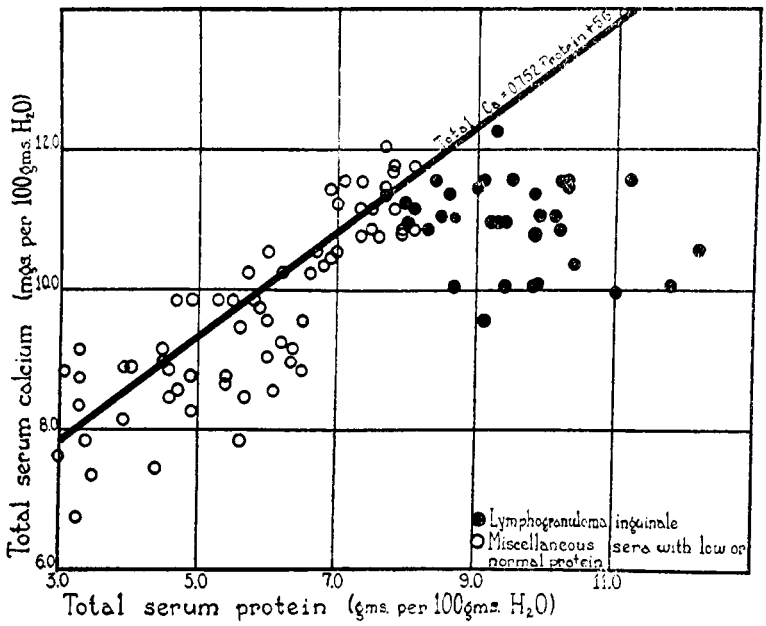

FIG. 1.

Our results on sera with normal protein, and protein low as a result of renal disease or malnutrition, show a direct proportionality between calcium and total protein which is in fair agreement with the mean empirical equation: Total Ca = 0.752 Protein + 5.6, given by McLean and Hastings.[4] This relation does not obtain in lymphogranuloma inguinale with hyperproteinemia (for values see [5]). Only cases with serum inorganic P of 2.5-5.0 mg. % have been used throughout.

Apart from some cases of multiple myeloma, where bone destruction co-exists, the total serum calcium does not rise in hyperproteinemia, but is maintained at normal levels.

The discrepant relation of calcium to protein in our cases of lymphogranuloma inguinale is not due to hyperphosphatemia, which was not present (Table I); or to a decrease in calcium not bound to protein, since the diffusible calcium fraction showed no convincing fall (Table I) and our patients show no evidence of tetany. The discrepancy results chiefly because despite the rise in total serum protein, there is no appreciable increase in the protein-bound calcium fraction.

TABLE I.
Total Protein and Calcium Fractions of the Serum in 5 Cases of Lymphogranuloma Inguinale (L.I.) and 3 Cases of Multiple Myeloma (M.M.).

		Serum							
							Diffusible Ca		
No.	Diagnosis	Total protein %	Alb. %	Glob. %	Euglob. %	Total Ca mg. %	mg. %	% of total Ca	Inorg. P mg. %
1.	L.I.	11.1	3.3	7.8	3.2	9.6	5.7	59	3.6
2.	L.I.	9.6	3.7	5.9	2.3	9.5	4.9	52	3.6
3.	L.I.	9.0	3.5	5.5	2.3	9.3	6.3	68	3.3
4.	L.I.	9.0	3.8	5.2	1.4	9.9	6.6	66	3.0
5.	L.I.	8.1	3.3	4.8	1.5	9.4	4.9	52	3.3
6.	M.M.	9.9	2.9	7.0	....	16.7	8.2	49	4.8
7.	M.M.	9.5	2.4	7.1	5.5	8.2	5.4	66	3.9
8.	M.M.	6.1	....	....	....	16.0	9.0	56	3.0

Mean % diffusible Ca/total Ca in normal sera = 61%.
No correction has been made either in normal or pathologic sera for Ca taken up by the collodion membranes.

The protein-bound calcium fraction fails to increase in hyperproteinemia due to lymphogranuloma inguinale for at least 2 reasons: 1. There is often some decrease in calcium bound to albumin since many of our cases show low normal or low albumin levels,[5] (Table I). Precisely how much lowering of the protein-bound calcium fraction is so effected cannot be determined because the calcium bound per gram albumin under the conditions obtaining in these sera is not known. Such approximations as can be made indicate that the fall in albumin in our cases is insufficient to account for the absence of definite hypercalcemia. 2. There remains the alternative that the protein-bound calcium fraction does not rise because a part (or all) of the globulin fraction does not bind a significant amount of calcium under the conditions existing in these sera. We suggest that this is the most important cause of the discrepant relation of calcium to protein in hyperproteinemia due to lymphogranuloma inguinale.*

* The discrepancy is a consequence of the fact that whereas *hypo*proteinemia is chiefly the result of loss of *albumin*, which binds an appreciable amount of calcium, *hyper*proteinemia is due to an increase in the *globulin* fraction, which is generally believed to bind significantly less calcium under the conditions obtaining in serum. Equation I in its present form does not allow for this difference. Expanding the term "m · protein" to "m_1 · albumin + m_2 · globulin" does not wholly resolve the difficulty, if, as is generally believed, m_2 is significant though small, since this would imply some increase in serum calcium with marked hyperglobulinemia. Our data on lymphogranuloma inguinale, however, reveal no upward trend whatever in total serum calcium levels as the serum globulin content increases (Fig. 1); *i. e.*, no significant amount of calcium is bound under these conditions either by the serum globulin fraction *in toto*, as Bendien and Snapper suggest[8], or by that

In multiple myeloma, as is well known, an absolute increase in the protein-bound calcium fraction may occur (Table I), but apparently only in cases presenting hypercalcemia, and then irrespective of whether or not the serum protein content is increased (Table I). It is suggested that an increase in protein-bound calcium in multiple myeloma with hyperproteinemia is a consequence not of the presence of increased globulin in the blood, but of dissolution of bone due to skeletal destruction. That the majority of cases of multiple myeloma presenting hyperproteinemia also exhibit hypercalcemia may mean only that myelomatosis severe enough to cause hyperproteinemia is likely to be extensive enough to produce widespread skeletal damage, with resulting hypercalcemia.

Conclusions. 1. Contrary to the prevailing view, hyperproteinemia (hyperglobulinemia) is not "a cause of" or "responsible for" hypercalcemia; it does not lead to abstraction of calcium from the bones either directly, or indirectly by upsetting the mass law relation[8] in the blood. In hyperproteinemia, even in the absence of hyperphosphatemia, there is no proportionality between total serum protein and serum calcium levels, and no inference as to the calcium content of the blood can be drawn from the total protein content.

2. Where hypercalcemia does occur in conjunction with hyperproteinemia, as in some cases of multiple myeloma, the calcium increase may well be due, not to hyperproteinemia, but to the complication of co-existent bone destruction by neoplastic tissue; like the hypercalcemia occurring occasionally with metastatic osteolytic carcinoma, in which serum protein levels are normal or low.[6, 9] The influx of Ca^{++} caused by bone destruction leads to an increase in protein-bound as well as in ionized calcium, with reëstablishment at higher levels of an equilibrium between these 2 fractions predictable by mass law considerations.[10] The absolute increase in the protein-bound calcium fraction, therefore, appears to be a *result* and not a cause of the hypercalcemia; the ratio diffusible Ca:total Ca remaining reasonably constant whether the serum protein is increased (multiple myeloma) or normal (hyperparathyroidism).

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part of the globulin fraction composing the increase in serum protein content (usually chiefly euglobulin).

In this study, only the diffusible calcium fraction was determined, no attempt being made to estimate the calcium ion concentration either directly or from mass-law considerations.[8]

[8] Bendien, W. M., and Snapper, I., *Biochem. Z.*, 1933, **260**, 105.

[9] Gutman, A. B., Tyson, T. L., and Gutman, E. B., *Arch. Int. Med.*, 1936, **57**, 379.

[10] McLean, F. C., Barnes, B. O., and Hastings, A. B., *Am. J. Physiol.*, 1935, **113**, 141.

3. An increased protein content of the serum is not essential to hold in solution such high calcium concentrations as occur in disease. Loeb and Nichols[11] showed that the calcium bound per gram protein is a function of the calcium concentration, the protein normally present in serum binding more calcium at increased than at normal calcium levels. This occurs, presumably, in hyperparathyroidism. In multiple myeloma with both hypercalcemia and hyperproteinemia, it seems not unlikely that most of the increase in calcium bound to protein is calcium bound by *albumin;* and little, if any, calcium is bound by the euglobulin increment responsible for the hyperproteinemia.

4. The discrepant relation of protein to calcium in hyperproteinemia is only a special case of the discrepant relation of protein to total base in hyperproteinemia. As was pointed out elsewhere,[5] the assumption that all of the serum globulin fraction in hyperglobulinemia binds as much base as the factor '*B* globulin' in general use[12] calls for, leads to the result that the sum of total determined acids appears to exceed the total base.

Methods. Serum calcium, inorganic phosphorus, total proteins and protein fractions were determined by methods described elsewhere.[5] The diffusible calcium fraction was estimated by ultrafiltration of 10-15 cc. serum samples through collodion sacs in a Simms apparatus[13]; positive pressures of 40-50 mm. Hg. were applied, the serum being maintained under normal CO_2 tensions. We are indebted to Dr. H. S. Simms and Mr. A. Stolman for their help.

11 Loeb, R. F., and Nichols, E. G., *J. Biol. Chem.*, 1927, **72**, 687.

12 Van Slyke, D. D., Hastings, A. B., Hiller, A., and Sendroy, J., Jr., *J. Biol. Chem.*, 1928, **79**, 769.

13 Simms, H. S., *J. Gen. Physiol.*, in press.