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An Empirical Regression Equation Relating Total Serum Calcium to Serum Albumin and Globulins.

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A number of empirical regression formulae have been proposed to express the relation between the level of total calcium in the serum and that of the serum proteins.[1] These equations relate total serum calcium to *total* serum protein and take the general form:

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

In the present study we have evaluated statistically the applicability of this general equation to our own data, consisting of 164 observations on 123 cases presenting a wide variety of changes in serum proteins.* The mean ratio: total serum calcium *calculated*/total serum calcium *observed* was calculated at different total protein levels, using values for m and b suggested by various investigators[1] and such other values as seemed appropriate.

A similar analysis was made of the general regression equations:

II. Total Ca $= m_1$. albumin $+ m_2$. globulin $+ b$ (where m_1, m_2 and b are constants).

and III. Total Ca $= m$. albumin $+ b$ (the special case where $m_2 = 0$).

As no constants for equations II and III could be found in the literature, values giving the best fit with our data were calculated.

Results. Equation I gives good agreement between calculated

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

* Our data include 27 observations on 21 cases of the nephrotic syndrome, with low albumin and normal or somewhat decreased globulin levels; 20 observations on 15 normal subjects; 50 observations on 39 cases of lymphogranuloma inguinale, 38 sera containing more than 8.0% total protein; 25 observations on 20 miscellaneous cases with hyperproteinemia not due to lymphogranuloma inguinale, multiple myeloma or cirrhosis; and 42 observations on 28 cases of hepatic cirrhosis, with low or normal albumin and normal or high globulin levels. Cases of extreme dehydration were not available for study.

All cases with a primary disturbance in calcium metabolism or with hyperphosphatemia were excluded. Multiple myeloma was not included because it is characterized by bone destruction (a primary disturbance in calcium metabolism). Cases of hypoproteinemia due to malnutrition or cachexia were excluded because the assumption of a constant Ca^{++} concentration—an assumption necessary because *total*, not protein-bound calcium values are used—is unwarranted in a condition where Ca^{++} may be so reduced that tetany results.

and observed total calcium values where the total protein is low or normal, but increasingly divergent results in hyperproteinemia if the means of the empirical values for *m* and *b* in the literature[1] are used (Table I). By decreasing *m*, *b* remaining constant (or by decreasing *b*, *m* remaining constant), calculated calcium values approach observed calcium values more closely in hyperproteinemia, but become too low in normal and nephrotic sera. It was not possible, by varying *m* or *b* or both in equation I, to obtain calculated calcium values in satisfactory agreement with observed calcium values for sera with normal globulin content and, at the same time, in hyperglobulinemia (Table I).

TABLE I.

Results of application of equations I, II, III and IV to our data, grouped according to total serum protein content. Representative mean ratios: $\frac{\text{total serum Ca calculated}}{\text{total serum Ca observed}}$ obtained by trial of various constants in these equations are shown. The ratios should be 1.00.

Formula Used	Constants Used	Mean Ratio $\frac{\text{Total Ca calculated}}{\text{Total Ca observed}}$ Total serum protein (gm. per 100 gm. H_2O)				
		3.1-6.0 (28 obs.)	6.1-8.0 (20 obs. normals)	6.1-8.0 (30 obs. patients)	8.1-10.0 (68 obs.)	10.1+ (18 obs.)
I.	Ca =.75 total protein +5.6	1.03	1.02	1.13	1.16	1.29
	Ca =.56 " " +5.6	0.94	0.89	0.99	1.00	1.09
	Ca =.75 " " +4.0	0.87	0.87	0.97	1.01	1.14
	Ca =.60 " " +6.0	1.01	0.95	1.06	1.07	1.18
II.	Ca =.83 albumin + .4 globulin +5.9	0.99	1.00	1.00	1.01	1.10
	Ca =.83 albumin +.25 globulin +6.0	0.96	0.97	0.99	0.99	1.01
III.	Ca =.80 albumin +7.1	1.01	1.00	0.95	0.96	0.93
	Ca =.80 " +7.2	1.02	1.00	0.96	0.97	0.94
	Ca =1.1 " +5.8	0.94	1.01	0.94	0.95	0.91
IV.	Ca =.80 albumin +.2 "globulin I" +7.0	1.00	0.99	0.99	0.99	1.00

In equation I, a common factor (*m*) is used for calcium bound to albumin + globulin (*i. e.*, total protein). The ratio: albumin/globulin does not remain constant, however, as the total serum protein content rises above or falls below normal levels; *hyper*proteinemia being due to an increase in the *globulin* fraction, *hypo*proteinemia chiefly to a fall in the *albumin* fraction.[2] The term '*m* . total protein', therefore, could be employed both in hyper- and hypoproteinemia only if the albumin fraction and the total globulin fraction

[2] Gutman, A. B., and Gutman, E. B., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 511.

bound approximately the same amount of calcium per gram. It appears from our analysis of equation I (Table I) that a common factor (m) cannot be so employed. At least in hyperglobulinemia, the amount of calcium bound per gram albumin appears to differ appreciably from that bound per gram total globulin under the conditions existing in these sera.

Equation II, unlike equation I, allows for differences in calcium bound per gram albumin and calcium bound per gram globulin and gives much better fit with our data. Nevertheless, it was not possible to find values for m_1, m_2 and b such that satisfactory agreement between calculated and observed calcium values was obtained both when serum globulin was normal and when it was definitely increased (Table I). These discrepancies result because the globulin fraction is itself heterogeneous; hyperglobulinemia being almost invariably due chiefly or wholly to increased euglobulin, as determined by Howe's method. A common factor (m_2) therefore could be used for the several serum globulins over the range of variation in total globulin content only if the several serum globulins bound approximately the same amount of calcium. The discrepancies encountered with equation II suggest that the several serum globulins bind different amounts of calcium under the conditions existing in these sera.[2, 3]

Equation III, which appears to imply that essentially all protein-bound calcium is bound to albumin, does not fit our data well in cases with marked hyperglobulinemia. The chief difficulty with this equation, however, is that b, which represents the amount of calcium not bound to protein and should be approximately 5.8 mg. per 100 gm. serum H_2O (the mean of determined values for diffusible calcium) must approach 7.0 if calculated values for total calcium are to approximate observed total calcium values. We interpret this discrepancy to mean that the constant 7.0 really is the sum of 2 constants; one of which (5.8) represents calcium not bound to protein, the other (approximately 1.2) represents calcium bound to protein but not to albumin. The latter calcium fraction is bound, presumably, to a globulin fraction ("globulin II"), and since it is a constant, remains of the same order of magnitude irrespective of increases in total globulin content. "Globulin II" roughly approximates, statistically, the pseudoglobulin II fraction as determined by Howe's method. The remaining globulin fraction, "globulin I", the globulin increment responsible for hyperglobulinemia (usually chiefly

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

euglobulin as determined by Howe's method) appears to bind so little calcium as to be insignificant except at high total globulin levels.

Conclusions. The above considerations suggest that the total serum calcium is composed of at least 4 fractions: 1. Calcium bound to and varying with albumin; 2. calcium bound to a globulin fraction, relatively constant in amount irrespective of the total globulin level; 3. calcium bound to another globulin fraction, varying with that globulin fraction, and though small, becoming significant at high total globulin levels; 4. calcium not bound to protein, relatively constant because cases with gross variations are excluded by definition. These fractions are represented in the following general regression equation:

IV. Total Ca $= m_1$. albumin $+ m_2$. "globulin II" $+ m_3$. "globulin I" $+ b$.

Where $b = 5.8 \pm 0.2$ mg. per 100 gm. serum H_2O, analysis of our data suggests that m_1 is of the order 0.7-0.9 mg. Ca per gram albumin; the product m_2 . "globulin II" is a constant of the order 1.0-1.5 mg. Ca per 100 gm. serum H_2O; m_3 is of the order 0.1-0.2 mg. Ca per gram "globulin I" where "globulin I" is defined arbitrarily as all globulin in excess of 3.0 gm. total globulin.

While the method does not warrant further precision of constants, it is of interest that a number of formulae based on equation IV may be devised which tend to obviate the systematic divergencies of equations I, II and III. Table I and Fig 1 illustrate the results where:†

V. Total Ca $= .80$ albumin $+ 7.0 + 0.2$. (total globulin—3.0).

Calcium is expressed in mg., albumin and globulin in gm. per 100 gm. serum H_2O. The constant 7.0 is the sum of b and m_2 . "globulin II". The last term is used only if the total globulin content exceeds 3.0 gm.

The fact that equation IV applies more generally to our data is regarded as support for the inferences drawn concerning calcium bound to the several protein fractions binding calcium and the approximate amounts so bound. It should be emphasized that no special significance is attached to the particular values used by us in equation V except as they are indicative of approximate orders of magnitude. We do not believe that a discrepancy between observed calcium values and those calculated by this or other equations neces-

† These constants are derived from sera of adults. The formula is not applicable where there is a primary disturbance in calcium metabolism, hyperphosphatemia or severe malnutritional hypoproteinemia, in which conditions gross variations in Ca^{++} occur. Appreciable variations in Ca^{++} concentration may occur in any wasting disease and constitute a source of error.

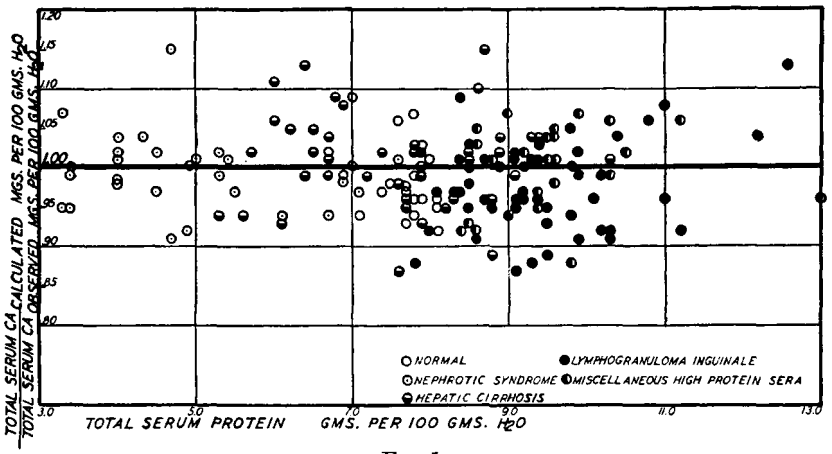

FIG. 1.

Correlation between observed Ca and Ca calculated by equation V for our data on 164 sera. The standard error of estimate is 0.575 mg. Ca. 65% of the mean ratios are within ±5% of 1.00; in 20 instances the mean ratio exceeded 1.05, of which 5 exceeded 1.10; in 32 instances the mean ratios were less than 0.95, of which 7 were less than 0.90. More symmetrical distribution but less satisfactory agreement with data in the literature is obtained if 7.1 is used as a constant instead of 7.0.

sarily implies the presence of a primary disturbance in calcium metabolism. As a prediction formula, equation V is of academic interest only, since total serum calcium may be determined directly with precision.

Methods. Serum calcium was determined by the Clark and Collip modification of the Kramer and Tisdall method. Serum protein was determined by difference, total N by the Kjeldahl technique, non-protein N by Folin's method with Nesslerization. Albumin and the globulin fractions were estimated by Howe's method, N being determined by the micro-Kjeldahl technique and titration. Determinations were made in duplicate, except some calcium determinations when insufficient serum was available. Corrections for specific protein volume were made[1]: 99.0—0.75 total protein.