and spleen were enlarged but the lungs were usually free from gross lesions. Caseation never occurred in the avian infections.

7201 P

State of Calcium in Protein-containing Fluids.*

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We have investigated the nature of the relationship between calcium and protein in blood serum and other protein-containing fluids, using the frog-heart method, previously described,[1] for direct estimation of calcium ion concentrations, and have accumulated evidence concerning the hypothesis[2] that there is a substance, like citrate, and other than protein, which binds calcium in serum and other fluids of the body. When purified electrolyte-free serum proteins (beef) are dissolved in solutions of approximately the electrolyte concentration of the serum, calcium combines with the protein in a manner analogous to that previously described for citrate.[3] The combination represents an equilibrium between protein, total calcium, and calcium ions, and this equilibrium may as a first approximation be described by the mass law equation

$$\frac{[Ca^{++}] \times [Prot^{=}]}{[CaProt]} = K \qquad (1)$$

in which

Ca^{++} = calcium ion concentration—mols. per kg. water.
† $Prot^{=}$ = base combining capacity of protein present at pH of solution, in equivalents $\times$ 0.5, less equivalents combined with calcium, per kg. of water
CaProt = calcium combined with protein expressed as mols of Ca per kg. water.
K = constant.

In logarithmic form this equation is written as $pCa^{++} + pProt^{=} - pCaProt = pK_{CaProt}$ in which p indicates the negative logarithm.

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1 McLean, F. C., and Hastings, A. B., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1344.

2 Greenwald, I., *J. Biol. Chem.*, 1926, **67**, 1.

3 McLean, F. C., and Hastings, A. B., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1136.

† The assumption, implied by the equation, that the base combining equivalents of protein behave as though they were divalent ions is purely empirical, in order to fit the data at hand.

TABLE I.
Calcium Ion Estimations.
Human Material.

Case	Fluid	Total Ca mM/Kg H_2O	Total Protein %	Ca^{++} — mM/Kg H_2O Calc.	Obs.	Diff.	Diagnosis
B	Serum	4.50	7.44	2.04	1.90	—.14	Unexplained Hypercalcemia
K	”	1.59	(6.17)	0.73	0.50	—.23	Uremia and Tetany
	”	1.81	(6.17)	0.83	0.60	—.23	Same—negative Trousseau
	”	1.28	(6.17)	0.59	1.15	+.56*	After recovery
R	”	2.40	6.31	1.12	1.15	+.03	Rickets
W	”	1.95	4.00	1.15	1.25	+.10	Nephrosis
	Pleural Fluid	1.62	1.80	1.25	1.25	—	”
	Ascitic Fluid	1.64	2.13	1.20	1.25	+.05	”
A	Edema Fluid	1.49	0.33	1.43	1.35	—.08	”
G	Serum	1.89	3.10	1.25	1.45	+.20	Amyloid nephrosis *in extremis*
S	”	2.16	5.11	1.12	1.20	+.08	” ”
M	”	2.80	7.73	1.15	1.10	—.05	Normal
	”	2.99	7.56	1.28	1.30	+.02	”

*Probably experimental error. Note: protein analyses not simultaneous.

When normal human serum is diluted with a solution containing approximately the electrolyte concentration of the serum, but without calcium, and when calcium is added to the various dilutions, the same relationship is found. From observations on both serum and purified serum proteins at pH 7.35 (approximately) a value of 2.22 has been derived for pK_{CaProt} when grams of protein are reduced to base combining capacity by use of the factors given by Van Slyke, Hastings, Hiller, and Sendroy.[4] Agreement of the values for pK_{CaProt} obtained from protein-containing fluids and from purified serum proteins indicates that it is unnecessary to postulate any appreciable amount of other calcium-binding substances in human serum.

Table I includes all observations on undiluted human material in which total calcium, total protein, and calcium ions have been determined upon the same specimens. In all cases the factor used for reducing grams of protein to base combining capacity at pH 7.35 is 0.243, which assumes a normal distribution of albumin and globulin. Further work may indicate the desirability of applying separate factors to these proteins.

These observations, while still few in number, include a wide range both of total calcium and total protein. Further work is required to establish the significance of such discrepancies between the calculated and observed calcium ion concentrations as are included in Table I.

Additional evidence that the frog-heart method is specific for ionized calcium, other conditions being equal, and that calcium bound to protein is physiologically inactive with respect to the frog-heart preparation, has been obtained from the observation that when citrate is added to solutions of purified proteins, or to serum, the values found for calcium ions satisfy simultaneously the mass-law equations for calcium citrate and for calcium proteinate.

For human material it is concluded that the protein-containing fluids may, from the standpoint of the state of calcium, be regarded as solutions of calcium proteinate, which dissociates as a weak electrolyte into calcium and protein ions, with an undissociated fraction of calcium proteinate, the degree of dissociation depending upon a mass-law relationship. At any given pH, therefore, the concentration of calcium ions is determined by the total calcium and the proteins present. Other substances binding calcium in human material, if any, must be in relatively low concentrations, unless as a result of unusual conditions.

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