A NOTE ON THE STATE OF CALCIUM IN HIGH PROTEIN SERUM

BY H. I. CHU AND A. B. HASTINGS

(From the Department of Biological Chemistry, Harvard Medical School, Boston)

(Received for publication December 27, 1937)

It has been found by Greenberg and Larson (1) that diffusible calcium and that combined with protein are quantitatively related according to the equation

\[
\frac{[\text{Prot.}]}{[\text{Ca Prot.}]} = 0.403 + \frac{5.80}{[\text{Ca}^{++}]} \cdot
\]

This is a special form of the mass law equation.

It has also been shown by McLean and Hastings (2) that the relation between the concentration of calcium ions \([\text{Ca}^{++}]\), and the concentration of calcium combined with serum proteins \([\text{Ca Prot.}]\), can be expressed by the mass law equation

\[
\frac{[\text{Ca}^{++}] \times [\text{Prot}]}{[\text{Ca Prot.}]} = K_{\text{Ca Prot.}} = 10^{-2.22},
\]

\[
\rho K_{\text{Ca Prot.}} = - \log K = 2.22.
\]

In the reported experiments of McLean and Hastings, their equation was found to be valid for predicting the calcium ion concentration from the total calcium and total protein when the sera contained 7.5 grams of protein per 100 cc. or less.

It has recently been shown by Gutman and Gutman (3) that serum from cases of hyperglobulinemia had less total calcium than one would have expected, if the calcium ion concentrations were normal and if the ionization constant of calcium proteinate \((\rho K_{\text{Ca Prot.}})\) had the value which it has in serum of normal protein concentrations. This raises the question as to whether protein which did not have the usual calcium combining power of normal serum albumin and globulin was present, or whether the ionization constant which holds for normal serum is different in high protein serum. The latter question could be answered by determining the value of \(\rho K_{\text{Ca Prot.}}\) in sera whose protein concentration had been increased. This communication presents the results of the determination of \(\rho K_{\text{Ca Prot.}}\) in such sera.

EXPERIMENTAL

Ten experiments were carried out, 5 on human serum, and 5 on horse serum. In two (Numbers 1 and 2), the serum was concentrated by ultrafiltration under a positive pressure of 180 mm. Hg. In the others, the serum was concentrated by evaporation to half its volume, and then dialyzed at constant volume against a balanced salt solution containing no calcium. Calcium chloride was subsequently added to give the desired calcium concentration. The sera, containing approximately 20 mM. HCO\(_3\) per liter, were equilibrated with a CO\(_2\) : O\(_2\) gas mixture until the pH equalled 7.35 to 7.40. Calcium ion concentrations were determined by the frog heart method (4); total calcium, by the method of Clark and Collip (5); albumin and globulin gasometrically, after separation according to Howe's technique (6). The water content was found to vary with the protein content according to the following equation

\[
[H_2O] \text{ gm. per 100 cc.} = 99.0 - 0.75 \quad [\text{Protein}] \text{ gm. per 100 cc.}
\]

and was, therefore, usually calculated and not directly determined. Concentrations have been expressed in units per kilo H\(_2\)O. Calculations of the concentrations of divalent protein ions per kilo of water \([\text{Total Prot.}^-]\) were made from the equation

\[
[\text{Total Prot.}^-] = 0.136 [\text{Albumin}] + 0.095 [\text{Globulin}],
\]

where albumin and globulin concentrations are expressed in terms of grams per kilo H\(_2\)O. It may be noted that

\[
[\text{Total Prot.}^-] = [\text{Prot.}^-] + [\text{Ca Prot.}]
\]

and that

\[
[\text{Ca Prot.}] = [\text{Total Ca}] - [\text{Ca}^{++}].
\]
From these data, the value of $K$ in the mass law equation can be calculated.

RESULTS AND CONCLUSION

The results of the determinations of the ionization constant expressed as $pK_{Ca Prot.}$ ($pK = - \log K$) are given in Table I. The mean of the determinations is 2.17 with a standard deviation of $\pm 0.06$. This compares favorably with the value of 2.22, standard deviation $\pm 0.07$, found for normal and low protein serum by McLean and Hastings. It will be seen that total protein concentrations as high as 121 grams per kilo of $H_2O$ do not lead to deviations in $pK$ which would be considered significant.

From these observations, it may be concluded, therefore, that the mass law equation adequately describes the relation between calcium ions and the calcium combined with protein, even though the protein concentration is increased to 60 per cent above normal. The results of Gutman and Gutman are, therefore, not to be accounted for by a failure of normal serum proteins in high concentration to combine with the predicted amount of calcium, but rather to the fact that they were dealing with sera containing abnormal proteins.

BIBLIOGRAPHY