THE EFFECT OF ALKALOSIS ON THE RELATIONSHIP 
BETWEEN SERUM CALCIUM AND PROTEIN IN VIVO*

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(Received for publication, September 26, 1940)

In the absence of definite experimental evidence, it has generally 
been assumed that the symptoms of tetany associated with in-
creases in serum pH are due to a decrease in the concentration of ionic calcium. However, by means of the frog heart method, McLean and Hastings (1) were unable to demonstrate any sig-
nificant change in calcium ions with change in pH. Because of 
the relatively narrow pH range in which these investigators 
worked, and the limited range of sensitivity of the method, no 
definite conclusions were offered, and the question of the effect of alkalosis on the capacity of protein to bind calcium was left open.

It has been abundantly confirmed that the concentrations of total calcium and protein in serum, in the absence of recognizable defects of calcium metabolism, bear a linear relationship to each other. This has been expressed by the regression equation, total Ca = a \times \text{total protein} + b, in which a and b are constants. Using data accumulated in the literature, McLean and Hastings (1) have demonstrated that the value for the intercept b in this equation is a close approximation of the concentration of ionic calcium as determined by their frog heart method, under nor-
mal conditions.

On the basis of these considerations, the relationship between total calcium and proteins in the serum of normal animals, and those with marked elevation of serum pH, was studied. It was hoped that by these means some idea would be gained as to the effect of alkalosis on the ability of serum protein to bind calcium

* Aided by grants from Child Neurology Research (Friedsam Founda-
tion) and the Fluid Research Fund of Yale University School of Medicine.
and the magnitude of the intercepts in the respective regression equations, which might give an approximation of the effect of alkalosis on the concentration of calcium ion.

**Procedure and Methods**

Cats were used as the experimental animals. They were fed on canned salmon and milk for at least 1 week before the experimental procedure. The method employed for producing alkalosis was as follows: The animals were injected intraperitoneally with a solution containing 50 gm. of glucose and 200 to 300 milliequivalents of sodium bicarbonate per liter, in amounts approximating 100 cc. per kilo of body weight. 5 hours later, a similar quantity of fluid was withdrawn. The serum changes resulting from this procedure have, in part, been previously described (2). These were an increase in pH and total CO₂, a decrease in chloride, and relatively little change in sodium. These changes persisted practically unchanged for at least 24 hours. At intervals of 2 hours, beginning with the withdrawal of the fluid from the peritoneal cavity, samples of blood were removed in amounts varying from 15 to 20 cc. A total of twenty-four blood samples was examined from six different animals. Of these, two animals survived 24 hours and were sacrificed, two animals died after 6 hours, and two animals died after 10 hours. The control observations were made on five animals. Of these, three animals were not treated in any way, and two animals were subjected to depletion of extracellular electrolytes as previously described (3). This resulted in marked hemoconcentration. By this procedure, and by the frequent withdrawal of fairly large quantities of blood, an adequate distribution of serum protein concentrations was obtained. A total of twenty-nine observations was made in the control group. All manipulations were carried out under nembutal anesthesia.

The blood was collected and allowed to clot under oil. The chemical analyses were carried out on the serum which was removed in the usual manner. Total protein was calculated from the nitrogen content determined by the Kjeldahl procedure. Total CO₂ was determined by the method of Van Slyke and Neill (4), phosphate by the method of Fiske and Subbarow (5), calcium by the method of Clark and Collip (6), and pH by Cullen's method (7) as modified for the Evelyn colorimeter.
Results

The data may be most conveniently presented by means of the statistical analysis. This is summarized in Table I. The average concentration of serum protein in both groups showed no significant difference. The range in the control animals was from 5.4 to 8.5 per cent, and in the alkalotic animals from 4.3 to 8.5 per cent. There was a significant decrease in the average calcium concentration in the alkalotic group, the individual values having a range from 6.3 to 10.4 mg. per cent. In the control group the calcium concentrations varied from 8.6 to 11.7 mg. per cent. There was a significant increase in the average value for phosphorus in the alkalotic group with a range in the individual values from 5.5 to 17.1 mg. per cent. In the control group phosphorus varied from 3.5 to 11.4 mg. per cent. The degree of alkalosis produced by the procedure employed may be seen from the average values for total CO₂ and pH.

Relationship between Protein and Calcium—The correlation coefficient between total protein and calcium in the serum of the normal animals was $0.55 \pm 0.13$, and the regression equation expressing this relationship $Ca = 0.62$ protein $+ 6.7$, in which the standard error of the regression coefficient is 0.18, and of the intercept 0.12. This agrees fairly closely with similar equations obtained in humans (8-10) and in dogs (11).

In the animals with alkalosis the correlation coefficient between protein and calcium was $0.58 \pm 0.17$, and the regression equation $Ca = 0.76$ protein $+ 2.61$, in which the standard error of the regression coefficient was 0.22, and of the intercept 0.20.
Effect of Alkalosis on Ca and Protein

Relationship between Phosphorus and Calcium—In the control group no correlation could be demonstrated between calcium and phosphorus. In the animals with alkalosis the correlation coefficient was $-0.34 \pm 0.19$, which statistically cannot be considered significant. When the data from both groups are combined, a significant negative correlation coefficient between calcium and phosphorus is obtained equal to $-0.56 \pm 0.10$. The regression equation expressing this relationship was $Ca = -0.26P + 11.3$, in which the standard error of the regression coefficient is 0.05, and of the intercept 0.17. The regression coefficient in this equation is very similar to that obtained by Peters and Eiserson (8) in human serum.

DISCUSSION

The type of alkalosis produced by the procedure employed in these experiments is essentially similar, as regards the resulting serum electrolyte configuration, to that following prolonged vomiting of gastric juice, or the administration of sodium bicarbonate to patients with impaired renal function. In the former, however, one frequently encounters reduced serum sodium concentrations as well. The effect of alkalosis on the concentration of serum calcium has been previously studied with equivocal results. Thus, Peters and Van Slyke (12) and Schmidt and Greenberg (13) in their reviews of this phase of calcium metabolism reach the conclusion that the alkalosis resulting from the administration of bicarbonate is not associated with a significant decrease in serum calcium. On the other hand, Stewart and Haldane (14), and recently McCance and Widdowson (15) in a case of pyloric obstruction, were able to demonstrate hypocalcemia, presumably secondary to an alkalosis due to excess alkali. Our experiments confirm the latter observations.

It will be noted in Table I that the variations in serum pH in both control and alkalotic groups are very small. In both groups about two-thirds of the individual observations are within 0.1 unit of the average for the group as a whole. The regression equations expressing the relationship between calcium and protein may therefore be considered as representing this relationship at two different but fairly constant levels of serum pH. A comparison of the two regression equations indicates: first, that the average
quantity of calcium bound to protein is slightly greater in the alkalosis group, although statistically this may not be significant; second, there is a significant decrease, in the alkalotic group, of calcium not combined to protein. This latter fraction of the total calcium represents primarily ionic calcium, although a small but appreciable quantity of bound but diffusible calcium is also included in this fraction (1).

The explanation for the reduction in ionic calcium during alkalosis is not clear. It will be noted that associated with this finding a significant hyperphosphatemia was demonstrated. This has been noted by others (15-17). Our data, however, add nothing directly bearing on the problem of the inverse relationship shown to exist between serum calcium and phosphorus under various conditions. The absence of a significant correlation between calcium and phosphorus in the individual groups is probably due to the lack of an adequate variation in serum phosphorus. When both groups were combined, the expected inverse relationship could be demonstrated.

McLean and Hastings (1), from their study of calcium ion concentrations in serum of various dilutions, concluded that the relationship of bound to ionic calcium follows the mass law equation describing the ionization of calcium proteinate. If it be assumed that \([\text{total Ca}] - [\text{Ca}^{++}] = [\text{calcium proteinate}],\) and \([\text{total protein}] - [\text{calcium proteinate}] = [\text{protein}^-],\) the mass law equation describing the ionization of calcium proteinate takes the following form

\[
[\text{Total Ca}] = \frac{[\text{Ca}^{++}]}{[\text{Ca}^{++}] + K} [\text{total protein}] + [\text{Ca}^{++}]
\]

As McLean and Hastings point out, the empirical calcium and protein regression equation is a special case of the mass law equation in which \([\text{Ca}^{++}]\) represents the intercept, and \([\text{Ca}^{++}] / ([\text{Ca}^{++}] + K),\) the slope. The rectilinearity of the regression equation is therefore dependent on the fact that, under the conditions in which the empirical regression equations cited in the literature were obtained, \([\text{Ca}^{++}]\) tended to be relatively constant. This was demonstrated by McLean and Hastings who found, by actual determination of \([\text{Ca}^{++}]\) in serum and protein containing body fluid containing widely varying concentrations of protein, a small
range in [Ca++] variability. Presumably, in our alkalosis experiments, the relatively constant value for serum pH was also associated with relatively little variability in [Ca++] in the group as a whole. From our data, we may calculate the pK of calcium proteinate for the control and alkalotic groups. The average values for calcium and protein are given in Table I, and expressed as moles per kilo of H₂O. The concentration of water is obtained from the formula, 99 − 0.75 protein. The factor for converting protein into base-combining equivalents was obtained from the data of Van Slyke, Hastings, Hiller, and Sendroy (18), with the average values for pH in Table I, and assuming an albumin to globulin ratio of 1.8 and a valence of 2 for the serum proteins. Calcium ion concentrations were obtained from the intercepts of the respective regression equations and expressed as moles per kilo of H₂O. In this fashion, pK values for calcium proteinate in the control and alkalotic groups were calculated as 1.96 and 2.37 respectively. In a similar manner, McLean and Hastings calculated the respective pK values from the calcium and protein regression equations given by five different investigators. These were found to vary from 1.97 to 2.28, averaging 2.12. The pK value for calcium proteinate determined by Hastings and McLean from their data on calcium ion concentrations in serum with the frog heart method was 2.22 ± 0.07. Considering the limited number of observations with the intercept in the regression equation as a measure of [Ca++] , our values for the pK of calcium proteinate cannot be considered as significantly out of line. It must therefore be concluded, tentatively, that our data on the relationship between serum calcium and protein in vivo, in the presence of marked elevations of serum pH, agree with the conclusions of Hastings and McLean that the behavior of these serum constituents may be described by a simple mass law equation as a first approximation.

SUMMARY

The relationship between calcium and protein in the presence of a marked degree of alkalosis was studied in cats.

A significant hypocalcemia due presumably to marked reduction in calcium ion concentration was demonstrated. Hyperphosphatemia was also found.
The relationship between calcium and protein was such as to agree with the conclusion of McLean and Hastings that this relationship may be described, as a first approximation, by a simple mass law equation yielding the ionization constant of calcium proteinate.

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