[CONTRIBUTION FROM THE BIOLOGICAL LABORATORY, COLD SPRING HARBOR, THE MEDICAL SERVICE OF DR. GEORGE BAEHR AND THE LABORATORIES OF THE MOUNT SINAI HOSPITAL, NEW YORK CITY, AND THE DEPARTMENT OF BOTANY, UNIVERSITY OF MINNESOTA]

# The Effect of Phosphate Buffers on the Electrical Mobility of Hemoglobin

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This communication deals with part of the data in a recent article by Davis and Cohn[1] entitled, "The Influence of *p*H and Ionic Strength on Electrophoretic Mobility." It is clear that the influence of ionic strength on the electric mobility of proteins at a given *p*H must embody two important effects: (1) the direct effect of the ions on the charge of the particle, (2) the effect of the ionic atmosphere on the electric mobility of a molecule of given charge. It has been shown[2,3,4,5] previously that the second effect, the effect of the ion atmosphere on the motion of the particle, could be accounted for in certain simple systems by some form of the Debye equation

$$u = u_0/(1 + \kappa r) \qquad (1)$$

(where $u$ is the electric mobility, $u_0$ the value of the mobility at infinite dilution, $\kappa$ the well-known function of ionic strength) when suitable corrections for shifts in the isoelectric point due to specific ion effects (of the first type) were considered. Davis and Cohn[1] have stated that their data are not satisfied by equation (1). In doing so they reject the methods previously employed by us[2–5] as applied to their data. It will be shown in this communication that the methods previously employed by us are applicable to the more complicated systems studied by Davis and Cohn.

The method employed by Davis and Cohn[1] to apply equation (1), as far as we can judge from their paper, implies that at a given value of the *p*H the net charge of the protein molecule is the same for all ionic strengths. This obviously is impossible, for an inspection of their data discloses very large shifts in the isoelectric point. Since the net charge is zero only at the isoelectric point, the complete family of curves indicates protein molecules having net charges of zero and finite values at any given *p*H in the range investigated.

To use our previous approach[2,3,4,5] it is necessary to make some assumption regarding the change in net charge with ionic strength. For uni-univalent electrolytes it is generally agreed that the influence of ionic strength on the shape of the net charge–*p*H curve is not very great.[6] Indeed, the change in shape may be neglected for small shifts in ionic strength. To obtain a reference point where the net charge is zero at all ionic strengths, we have previously shifted all curves to the same isoelectric point, keeping the curves parallel along the mobility axis. That these assumptions also may apply to systems containing phosphate buffers is demonstrated by treatment of the data of Davis and Cohn which follows.

Without any assumptions, except those inherent in the theory itself, we may apply the Debye theory by multiplying all the mobilities by the expression $(2 + \kappa r)$, to calculate the value of the mobility at zero ionic strength for a molecule of the same net charge. The empirical function $(2 + \kappa r)$ is used here because it gives essentially the same results ($\mu = 0.02$ to $0.15$) as the theoretical functions derived by Gorin,[5] in which the asymmetry of the protein and the finite size of the other ions in the solution are considered.[5,7] The value of $r = 27$ Å. was employed.

In Fig. 1 the data of Davis and Cohn[1] on the effect of phosphate buffers on the electric mobility of hemoglobin are plotted as smooth curves. The dashed lines passing through the same isoelectric points are corresponding values of the mobility multiplied by $(2 + \kappa r)$. It may be observed that all of the dashed curves are closely parallel to one another, any deviation being at the limit of experimental error. This is made more evident by an inspection of Fig. 2, in which all of the data have been plotted by shifting the parallel curves to the same isoelectric point. It therefore appears that the assumptions we have heretofore employed for proteins in solutions of uni-univalent electrolytes, contrary to the view of Davis and Cohn,[1] may be applied to their data in the case

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(1) B. D. Davis and E. J. Cohn, THIS JOURNAL, **61**, 2092 (1939).

(2) H. A. Abramson, *J. Gen. Physiol.*, **15**, 575 (1932); **16**, 593 (1933).

(3) H. A. Abramson, "Electrokinetic Phenomena," The Chemical Catalog Co., Inc., New York, 1934.

(4) L. S. Moyer and J. C. Abels, *J. Biol. Chem.*, **121**, 331 (1937). L. S. Moyer and H. A. Abramson, *ibid.*, **123**, 391 (1938).

(5) H. A. Abramson, M. H. Gorin and L. S. Moyer, *Chem. Rev.*, **24**, 345 (1939).

(6) R. K. Cannan, "Symposia on Quantitative Biology," Cold Spring Harbor, 1938, p. 1.

(7) M. H. Gorin, *J. Chem. Phys.*, **7**, 405 (1939).

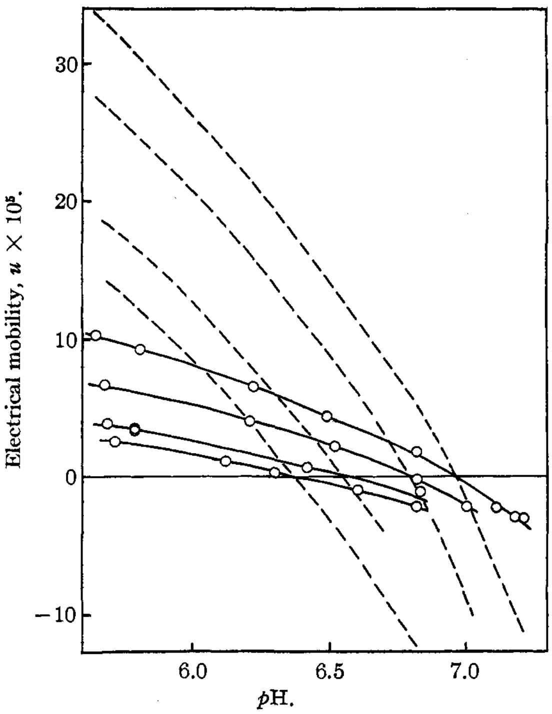

Fig. 1.—The data of Davis and Cohn at ionic strengths 0.02, 0.05, 0.10, 0.15, respectively, from top to bottom, are represented by the solid curves. The dashed lines represent their data multiplied by the function ($\kappa r + 2$).

under discussion. If the incomplete function, $(1 + \kappa r)$, is employed the agreement is not quite as good. This confirms similar observations made for proteins in simpler buffer solutions.[2,3,4,5]

In conclusion, with a view to avoiding confusion, it should be pointed out that the equation finally adopted by Davis and Cohn[1] (employing shifts in the isoelectric point)

$$u = u_0 - \beta\sqrt{\mu}/(1 + \sqrt{\mu}r/3) \qquad (2)$$

was in reality used as an empirical form of the equation[8]

$$u = u_0/(1 + \kappa r) \qquad (1)$$

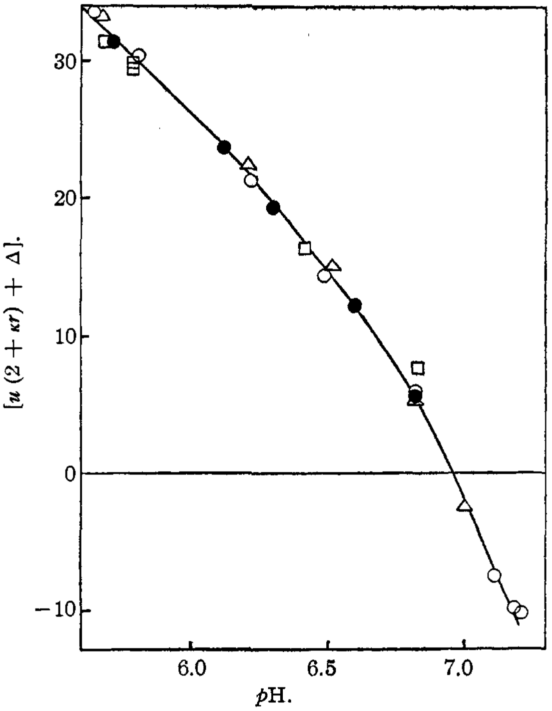

Fig. 2.—The recalculated data of Davis and Cohn have been brought to the same isoelectric point (that for ionic strength 0.02). The following symbols ○, △, □, ●, are for the data at ionic strengths 0.02, 0.05, 0.10 and 0.15, respectively. The values of Δ are 0, 6.4, 13.0, 17.7, respectively.

## Summary

Variations of the ionic strength of a buffer at constant $p$H can affect the electric mobility of a protein in two ways: (1) a change in ion atmosphere will occur, (2) a change in the charge of the protein might occur due to specific interaction with the ions of the buffer. It is shown that when both effects are taken into consideration in a way that has previously been applied to proteins in buffer systems containing only uni-univalent ions the recent data of Davis and Cohn on the electric mobility of hemoglobin in phosphate buffers are reasonably explained.

RECEIVED OCTOBER 16, 1939

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[8] Compare D. A. MacInnes, "The Principles of Electrochemistry," Reinhold Publishing Corp., New York, 1939, page 325, with Abramson,[2] p. 104.