activity predicted higher activity for compound 5 than for compound 1 and for compound 6 than for compound 2, but no substantial differences are observed. It must be concluded therefore that either the hypothesis is incorrect and that inductive effects are insignificant or that other equally important compensating factors are in effect.

On the basis of polarity of ring substituted benzyldimethylalkylammonium chlorides as predicted from dipole moments of the corresponding substituted toluene a direct relationship of polarity of substituents with CMC and biological activity has been suggested.[4] In a series of substituted benzyldimethyldodecylammonium chlorides in which the corresponding toluene derivatives possessed dipole moments ranging from 0.4 to 4.40 the CMC range is from 0.28 to 3.6 $\times 10^{-3}$ M. Comparing these observations with those of the present study a wider CMC range (1.4 to 9.0 $\times 10^{-3}$ M) is noted in a series of compounds in which the cyclic substituents possess very low polarity as predicted from dipole moments of the corresponding hydrocarbons. Considering the facts which are available it is suggested that simple steric effects are a major factor in determining the tendency toward micelle formation and the biological activity of cationic surface active agents. These phenomena are perhaps a function of the ability of the molecules to undergo close packing which in turn influences the size of the micelle or the extent of interaction with the bacterial surface.

Acknowledgment.—The authors are indebted to Mr. I. Katzman for the statistical analysis of the bacteriological data and to Mr. A. E. Brake for the spectrophotometric analyses.

CHICAGO 9, ILLINOIS

(4) S. Ross, C. E. Kwartler and J. H. Bailey, *J. Colloid Sci.*, **8**, 385 (1953).

[CONTRIBUTION NUMBER 30 FROM THE DEPARTMENT OF BIOPHYSICS, FLORENCE R. SABIN LABORATORIES, UNIVERSITY OF COLORADO MEDICAL CENTER]

Binding of Salt Ions by Bovine γ-Pseudoglobulin[1]

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At *p*H values within its isoelectric zone, bovine γ-pseudoglobulin aggregates at very low salt concentrations, presumably due to association of molecules of opposite charge. In contrast, at *p*H 3.1 the protein aggregates at high but not low NaCl concentrations and at lower concentrations of other supporting electrolytes. The effectiveness of univalent anions in aggregating the protein at *p*H 3.1 increases in the order Cl, Br, NO_3 and ClO_4. In terms of equivalent concentration sulfate appears to fall between bromide and nitrate at high concentration, but is more effective than nitrate at low concentration. Interpretation of these results in terms of binding of anions by the protein is supported by electrophoretic measurements. The mean isoelectric *p*H value of the protein decreases on substitution of Br, NO_3 and ClO_4 for Cl in the buffer solvent, the effectiveness of the substituting anions increasing in the mentioned order. Sulfate also decreases the isoelectric point of the protein when substituted for chloride in the buffer, but the relative effectiveness of this anion as compared with the univalent anions has not been established.

Introduction

Unpublished data obtained in this Laboratory show that whereas the major portion of bovine γ-pseudoglobulin sediments as a single boundary at a rate independent of the *p*H over the range 7.4 to 4.2, the sedimentation constant of the protein decreases by about 10% between *p*H 4.2 and 3.5 and then once again becomes independent of *p*H over the range 3.5 to 2.2. In neutral solutions the sedimentation behavior of γ-globulin is independent of salt concentration over the range 0.02 to 1 M NaCl, whereas at *p*H 3.1 the sedimentation behavior is strongly dependent on salt concentration. The sedimentation behavior of the protein in acidic solutions is also dependent upon the anionic nature of the supporting electrolyte. Experiments designed to help elucidate the nature of the structural changes which occur in acidic solutions and give rise to the observed changes in sedimentation behavior are still in progress. However, the authors wish to report the results of some of these experiments which indicate that the sedimentation behavior of γ-globulin in acidic solutions can be modified appreciably by binding of various anions.

Experimental

Material.—The bovine γ-pseudoglobulin used in these experiments was the water-soluble fraction of Armour Fraction II of Bovine Plasma. A solution containing 3 g. of Fraction II/100 ml. was dialyzed exhaustively against many changes of cold, distilled water; the water-insoluble fraction removed by centrifugation and discarded; and the water-soluble fraction stored in salt-free solution at 2°. The sedimentation behavior of the protein as determined at ionic strength 0.1 and at *p*H 6.8 and 3.1 did not change during several months of storage.

Ultracentrifugal Analysis.—Sedimentation velocity experiments were performed in the Spinco Model E electrically driven ultracentrifuge.[2] A sedimentation cell with a Kel-F centerpiece was used at *p*H 3.1. Runs were made at room temperature on 1.1–1.3% protein solutions at 59,780 r.p.m. The temperature of each run was taken as one degree less than the mean of the rotor temperature at the beginning and end of the run.[3] Distances from the reference line to the meniscus and to the position of the maxima of the schlieren peaks and the areas under the peaks were measured on projected tracings of the photographic records of the sedimenting boundaries. Allowance was made for the stretching of the rotor.[4] Sedimentation constants were

(1) This investigation was supported in part by a research grant from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, Public Health Service; and in part by an institutional grant from the Damon Runyan fund and the American Cancer Society.

(2) Specialized Instruments Corporation, Belmont, California.

(3) D. F. Waugh and D. A. Yphantis, *Rev. Sci. Instr.*, **23**, 609 (1952).

(4) G. L. Miller and R. H. Golder, *Arch. Biochem. Biophys.*, **36**, 249 (1952); J. F. Taylor, *ibid.*, **36**, 357 (1952).

TABLE I

EFFECT OF CONCENTRATION AND NATURE OF SUPPORTING ELECTROLYTE ON ULTRACENTRIFUGAL COMPOSITION OF BOVINE γ-PSEUDOGLOBULIN[a]

Part I. Sedimentation at *p*H 6.9–7.4

Supporting electrolyte	Distilled water		0.005 *M*		0.01 *M*		0.02 *M*		0.05 *M*		0.1 *M*		0.3 *M*	
	%	*S*	%	*S*	%	*S*	%	*S*	%	*S*	%	*S*	%	*S*
NaCl	77	6.90	78	6.84	84	6.87	89	6.65	85	6.58	90	6.44	91	6.53
	15	10	22	10	16	10	11	9.7	11	9.3	10	9.4	9	9.7
	5	19							4	12				
	3	31												
$NaClO_4$											89	6.62		
											11	9.1		

Part II. Sedimentation at *p*H 3.1 (Uni-univalent electrolytes)[b]

	0.02 *M*		0.05 *M*		0.1 *M*		0.2 *M*		0.3 *M*	
	%	*S*	%	*S*	%	*S*	%	*S*	%	*S*
NaCl[c]	100	4.44	100	5.54	99–96	5.63	83	6.02	82	6.23
					1–4	7.5	14	8.8	13	9.5
							3	12	5	12
NaBr							80	6.80	41	7.20
							7	9.8	5	10
							13	15[d]	8	14[d]
									46	42
$NaNO_3$	100	4.89			73	6.33	36	6.75		
					9	8.8	7	10		
					18	19	4	13		
							53	44[d]		
$NaClO_4$			64	6.58	20	7.30				
			15	9.6	3	11				
			8	12	9	14				
			13	17	68	120[d]				

Part III. Sedimentation at *p*H 3.1 (ammonium sulfate)[e]

	0.009 *M*		0.017 *M*		0.033 *M*		0.1 *M*	
	%	*S*	%	*S*	%	*S*	%	*S*
$(NH_4)_2SO_4$	91	6.03	80	6.41	70	6.72	66	6.96
	9	8.7	12	9.2	18	9.6	22	9.9
			8	12	12	14	12	13

[a] Protein concentration: 1.1–1.3 g./100 ml. [b] Protein solution was aged one hour before sedimentation. *p*H was adjusted with HCl except in the case of $NaNO_3$ where HNO_3 was used. [c] The protein had the same sedimentation constant in KCl–HCl, 0.02 and 0.1 *M*, and in NH_4Cl–HCl, 0.1 *M*, as in NaCl–HCl solutions at the same *p*H and salt concentrations. [d] Heterogeneous. [e] The *p*H was adjusted with H_2SO_4, and the solutions aged one hour at room temperature before sedimentation.

computed by the method of Cecil and Ogston[5] and were corrected to the standard conditions of pure water at 20° as the hypothetical solvent. The partial specific volume was taken as 0.745. Values of the sedimentation constants are reported in Svedberg units ($S = 1 \times 10^{-13}$/sec.). The areas under the peaks were corrected in the usual manner for dilution in the sector shaped cell.

Electrophoretic Analysis.—The Perkin–Elmer Tiselius apparatus, fitted with the current-regulating power supply described by Swingle[6] and a potentiometer to measure the voltage drop across a standard resistance placed in series with the cell, were used in these experiments. A portion of the stock solution of γ-globulin was diluted to 1 g. protein/100 ml. and dialyzed at 4° for 48 hours against two portions of buffer before analysis. The *p*H values were measured at 0°.

Results

Sedimentation Studies.—Sedimentation experiments were carried out on bovine γ-pseudoglobulin in solutions of various salt concentrations at nearly neutral reaction and at *p*H 3.1. Several different types of supporting electrolytes were employed. Portions of the salt-free stock solution of γ-globulin were diluted and adjusted to the desired salt concentrations by the addition of appropriate quantities of concentrated salt solution. In the case of experiments carried out at *p*H 3.1, the *p*H was then adjusted by addition of acid. The solutions were aged for one hour at room temperature before sedimentation. The results of representative experiments are presented in Table I. At nearly neutral reaction the sedimentation behavior of γ-globulin is independent of the salt concentration over the range 0.02 to 1 *M* NaCl. The sedimentation patterns reveal that about 90% of the protein sediments as a single boundary with a sedimentation constant slightly greater than that found previously for human γ-pseudoglobulin[7,8] and the remainder contains at least one more rapidly sedimenting boundary. At lower salt concentrations the major boundary sediments at a significantly greater rate than at the higher concentrations, and the relative

(5) R. Cecil and A. G. Ogston, *Biochem. J.*, **43**, 592 (1948).

(6) S. M. Swingle, *Rev. Sci. Instr.*, **18**, 128 (1947).

(7) J. R. Cann, THIS JOURNAL, **75**, 4213 (1953).

(8) The value of the extrapolated sedimentation constant of bovine γ-pseudoglobulin is 6.84 *S*. In the case of human γ-pseudoglobulin the value, after an approximate correction for the change in rotor temperature during acceleration and deceleration, is 6.7 *S*.

proportion of the faster sedimenting boundary increases. In distilled water the sedimentation patterns are rather complex and show 77% of a major boundary with a sedimentation constant of 6.90 *S* and three minor ones: 15%, 10 *S*; 5%, 19 *S*; and 3%, 31 *S*. As reported previously[9] human γ-pseudoglobulin behaves similarly at very low salt concentrations. The more rapidly sedimenting boundaries probably correspond to aggregated protein[10] molecules formed by association of globulin molecules of opposite charge.[11] The increased rate of sedimentation of the major boundary at very low salt concentrations might indicate that under these conditions the major boundary represents an equilibrium mixture of monomeric and aggregated protein.[13] The effect of very low salt concentration on sedimentation behavior is reversible.

Essentially the same sedimentation behavior was observed in 0.1 *M* $NaClO_4$, *p*H 6.9, as in 0.1 *M* NaCl, *p*H 6.7–7.2.

The sedimentation behavior of γ-globulin at *p*H 3.1 is considerably different from its behavior at nearly neutral reaction. Consider, for example, the ultracentrifugal composition of the protein in NaCl solutions. At *p*H 3.1, 0.1 *M* NaCl, 99–96% of the protein sediments as a single boundary with a sedimentation constant about 10% lower than that of the major boundary in patterns obtained at *p*H 6.7–7.2. The sedimentation behavior at *p*H 3.1 is very sensitive to the concentration of the supporting electrolyte. Thus, the sedimentation constant of the protein decreases continuously from a value of 5.63 to 4.44 *S* as the NaCl concentration is varied from 0.1 to 0.02 *M*, respectively.[15] At higher salt concentrations, 0.2 to 0.4 *M*, the sedimentation patterns show about 82% of a major boundary with a sedimentation constant which is 8–16% greater than observed in 0.1 *M* solution, and 18% of more rapidly sedimenting boundaries. At a salt concentration of 0.3 *M* the protein solutions assume a bluish hue, presumably due to Rayleigh scattering, when aged at room temperature, an indication that the faster sedimenting boundaries correspond to aggregated protein. The ultracentrifugal composition of the protein also changes on aging. Thus, a sample aged for one hour at room temperature contained 82% of a 6.23 *S* component; 13%, 9.5 *S*; and 5%, 12 *S*. After aging for an additional 8 hours at room temperature the protein contained 36% of a 6.38 *S* component; 4%, 9 *S*; 10%, 15 *S*; and 50%, 34 *S*. Even in 0.1 *M* NaCl aggregation of the protein proceeds very slowly at room temperature.

The sedimentation behavior at *p*H 3.1 was also found to be quite sensitive to the nature of the anion of the supporting electrolyte (the sedimentation behavior of the protein was the same whether NaCl, KCl or NH_4Cl was used as supporting electrolyte). In Table I the results of sedimentation experiments carried out using NaBr, $NaNO_3$, $NaClO_4$ or $(NH_4)_2SO_4$ as the supporting electrolyte are compared with those using NaCl. It will be noted that these electrolytes are more effective in aggregating[16] the protein than is NaCl. For example, the protein is aggregated to the extent of 18% in 0.3 *M* NaCl, whereas in 0.3 *M* NaBr it is aggregated to the extent of 59%. Also, the size of the aggregates is greater in the latter case. Again, compare the ultracentrifugal composition of the protein in 0.05 *M* NaCl and 0.05 *M* $NaClO_4$. In the first case the protein sediments as a single boundary with a sedimentation constant of 5.54 *S*, while in the second case the ultracentrifugal composition is 64%, 6.58 *S*; 15%, 9.6 *S*; 8%, 12 *S*; and 13%, 17 *S*. In $(NH_4)_2SO_4$ solutions the protein was aggregated to some extent even at a salt concentration as low as 0.009 *M*. The effectiveness of univalent anions in aggregating the protein increases in the order Cl, Br, NO_3 and ClO_4. In terms of equivalent concentration the sulfate ion appears to fall between bromide and nitrate at the highest concentration studied, but is more effective than nitrate ion at the lowest concentration.

Another interesting feature of these data is that the sedimentation constant of the slowest sedimenting boundary in NaBr, $NaNO_3$, $NaClO_4$ and $(NH_4)_2SO_4$ solutions at *p*H 3.1 is greater than in NaCl solutions of the same salt concentrations and *p*H. It does not seem likely that this is due entirely to a secondary charge effect,[14] and the authors are inclined to believe that this difference is in part at least a reflection of a difference in protein structure in the various electrolytes. In those cases where the sedimentation constant of the slowest boundary is actually greater than observed in NaCl and $NaClO_4$ solutions at nearly neutral reaction, one is tempted to speculate whether the boundary may not represent an equilibrium mixture of monomeric and aggregated protein.

An interesting observation made in the experiments carried out in acidic solution is that a rigid gel was found adhering to the bottom of the centrifuge cell at the end of each run. This suggests that at sufficiently high protein concentrations gelation can occur in acidic media.

Electrophoretic Studies.—The electrophoretic mobilities of bovine γ-pseudoglobulin near the isoelectric *p*H were measured in sodium cacodylate cacodylic acid buffer solvents which were 0.02 *M* in sodium cacodylate and contained sufficient NaCl, Na_2SO_4, NaBr, $NaNO_3$ or $NaClO_4$ to give a total ionic strength of 0.1. The descending electropho-

(9) J. R. Cann, THIS JOURNAL, **75**, 4218 (1953).

(10) Solutions of γ-pseudoglobulin in distilled water, 1.6–2.7 g. protein/100 ml., have a bluish hue presumably due to Rayleigh scattering.

(11) These experiments were carried out at *p*H values within the isoelectric zone of γ-globulin so that the solutions contained both positively and negatively charged globulin molecules.[12]

(12) R. A. Alberty, THIS JOURNAL, **70**, 1675 (1948); *J. Phys. Colloid Chem.*, **53**, 114 (1949); and J. R. Cann, R. A. Brown and J. G. Kirkwood, THIS JOURNAL, **71**, 2687 (1949).

(13) The primary charge effect, which one would expect to be important in distilled water, would reduce the rate of sedimentation of the protein.[14] This is opposite to the observed effect.

(14) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Clarendon Press, London, 1940, p. 23.

(15) That the protein sediments with the same sedimentation constant in KCl–HCl solutions at *p*H 3.1, salt concentrations of 0.1 and 0.02 *M*, as in NaCl–HCl solutions at the same *p*H and salt concentration, indicates that the dependence of the sedimentation constant on salt concentration is not due to a primary charge effect.[14] The primary charge effect would be expected to be minimal in solutions of KCl (personal communication from Professor John G. Kirkwood).

(16) Many of the protein solutions which contained rapidly sedimenting components had a bluish hue, the intensity of which increased with the length of time the solutions were aged at room temperature.

retic patterns were unsymmetrical and somewhat diffuse, while the rising patterns showed a poor resolution into two peaks. The mean mobilities were calculated from the descending patterns using the first moment of the gradient curves. The results are presented graphically in Fig. 1, where the mean mobilities are plotted against the *p*H of the buffers. The mean isoelectric *p*H values, *p*I, of the protein in the different media were determined from these plots and are given in the legend for the figure. The isoelectric *p*H value for the protein decreased on substitution of SO_4, Br, NO_3 and ClO_4 for Cl. These effects are quite large, *e.g.*, the mean *p*I value decreases by about 1.7 *p*H units on substitution of perchlorate for chloride, but such strong dependence of *p*I on buffer composition is perhaps not too surprising. Cann, Brown and Kirkwood[17] have reported differences as great as 0.87 *p*H unit in the isoelectric points of Armour bovine γ-globulin and its fractions in chloride–cacodylate and phosphate buffers of the same ionic strength.

Discussion

The structural changes responsible for the decrease in the sedimentation constant of γ-globulin between *p*H 4.2 and 3.5 and for the strong dependence of the sedimentation behavior of the protein on salt concentration at *p*H 3.1 are as yet unknown. However, certain interesting conclusions can be drawn from the results of the experiments described above. In solutions at *p*H values within the isoelectric zone of γ-globulin, the protein aggregates at very low salt concentrations, presumably due to association of molecules of opposite charge. In contrast, at *p*H 3.1 the protein aggregates at high but not low NaCl concentrations and at lower concentrations of other supporting electrolytes. It would appear that as a result of structural changes incurred on exposure to acidic media similarly charged globulin molecules can aggregate in these media. Since the molecules carry a large positive charge and thus repel each other, a high concentration of NaCl is required to shield the charges sufficiently by means of a double layer to allow aggregation to occur at an appreciable rate.[18] Binding of anions by the protein would reduce its net positive charge. In that case one would expect that a lower concentration of supporting electrolyte would be required in order to shield the charges sufficiently to give an appreciable rate of aggregation. Thus, differences in binding of anions, presumably by the positively charged amino groups of the protein, would seem to afford an explanation of the differences in the sedimentation behavior of γ-globulin solutions of different supporting electrolytes at *p*H 3.1.

The interpretation of the results of the sedimentation experiments in terms of anion binding is supported by the electrophoretic measurements. The observed changes in the mean isoelectric point of γ-globulin produced by changes in buffer composition at constant ionic strength is probably to be ascribed

(17) J. R. Cann, R. A. Brown and J. G. Kirkwood, *J. Biol. Chem.*, **181**, 161 (1949).

(18) E. J. W. Verwey and J. Th. G. Overbeek, "Theory of the Stability of Lyophobic Colloids," Elsevier Publishing Co., Inc., Houston, Texas, 1948, Chap. XII.

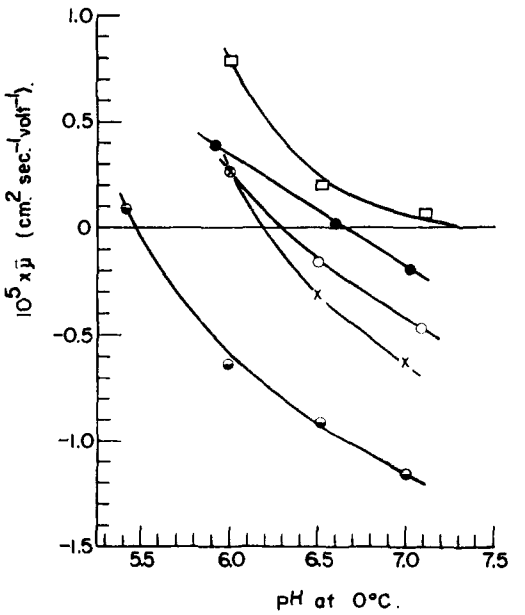

Fig. 1.—Mean electrophoretic mobilities, $\bar{\mu}$, of bovine γ-pseudoglobulin at *p*H values near the mean isoelectric *p*H in different buffer solvents at ionic strength 0.1: □, cacodylate–chloride, *p*I = 7.2; ●, cacodylate–sulfate, *p*I = 6.63; ○, cacodylate–bromide, *p*I = 6.28; X, cacodylate–nitrate, *p*I = 6.18; ◒, cacodylate–perchlorate, *p*I = 5.46.

to differences in binding, by the protein, of salt ions, especially anions. One would expect that the more strongly bound a given anion, the more effective it will be in decreasing the isoelectric point of the protein. The effectiveness of the several univalent anions in decreasing the isoelectric point when substituted for Cl in the buffer increases in the order[19] Br, NO_3 and ClO_4 which is also the order of their effectiveness in aggregating the protein at *p*H 3.1. Judging from the changes in the net charge on the protein produced by substitution of a given anion for chloride in the buffer at constant *p*H, it would appear that the extent of binding of the substituting anions by γ-globulin is of the same order as shown by serum albumin at the same electrolyte concentrations (see Appendix). Bovine γ-globulin has been found to have little affinity toward methyl orange under conditions where bovine serum albumin binds this anion extensively.[20] However, these were carried out at much lower concentrations of the anions than those used in the present study.

It is of interest to compare the effect of anions on the physical properties of several proteins in acidic solutions. As in the case of γ-globulin, conalbumin sediments as a single boundary in solutions of low NaCl concentration at *p*H 3 but is aggregated at high salt concentrations.[21] In acidic solutions

(19) Although the electrophoretic data indicate that sulfate is bound by γ-globulin, they do not establish its relative effectiveness in decreasing the isoelectric point of the protein, since the equivalent concentration of this anion was less than that of the univalent anions; nor do they establish the relative extent of binding of the divalent sulfate and univalent chloride.

(20) I. M. Klotz and F. M. Walker, *J. Phys. Colloid Chem.*, **51**, 666 (1947); I. M. Klotz and J. M. Urquhart, THIS JOURNAL, **71**, 1597 (1949).

(21) J. R. Cann and R. A. Phelps, *Arch. Biochem. Biophys.*, **52**, 48 (1954).

of Na_2SO_4 the protein is highly aggregated even at very low salt concentrations. Unpublished data indicate that substitution of NaBr, $NaNO_3$ and $NaClO_4$ for NaCl as the supporting electrolyte produces changes in the sedimentation behavior of conalbumin at *p*H 3 similar to those reported herein for the case of γ-globulin. Using the methods of equilibrium dialysis and sedimentation, Fredericq and Neurath[22] have shown that the binding of thiocyanate by insulin decreases the extent of molecular dissociation of this protein in acidic solution. The effect of iodide is qualitatively the same as that of thiocyanate. In contrast, molecular dissociation of insulin proceeds further in the presence of dihydrogen phosphate than in the presence of chloride. These results were interpreted in terms of the effect of ion binding on the net charge of the protein. Weber[23] has shown that the polarization of fluorescence of bovine serum albumin conjugate is independent of *p*H between 4 and 9, but falls rapidly outside this region due to reversible changes in the relaxation time of the rotation of the protein molecule. The polarization of fluorescence in acidic solutions increases upon addition of neutral salts. With increasing concentration of most salts the polarization tends asymptotically to the value observed in neutral solution. The effectiveness of various anions increases in the order Cl, Br, SO_4, NO_3, CNS, p-$CH_3 \cdot C_6H_4 \cdot SO_3$, and is attributed to changes in the charge on the protein due to anion binding. It would appear that the binding of anions has a pronounced effect on the structure of a variety of proteins in acidic solution.

Appendix

Changes in the net charge on the protein produced by changes in buffer composition were estimated from the observed mobility changes using a theoretical value for the ratio of mobility to net charge computed with the aid of the Debye–Hückel–Henry[24] theory. The assumptions involved in such calculations are discussed by Longsworth and Jacobsen.[25] Since a theoretical rather than an experimental value for the ratio of mobility to charge was used, the calculated change in net charge on the protein may have been underestimated by as much as 30%.[25] Let us consider the results of one such calculation. The difference in the net charge of the protein in perchlorate- and in chloride-rich buffers, Δz, was estimated to be about -7 at *p*H 7.0 and about -8 at *p*H 6.0. Interpretation of Δz in terms of a difference in binding of perchlorate and chloride is complicated by possible differences in the extent of binding of protons, sodium and cacodylate ions, and cacodylic acid by the protein in the two buffers. However, it seems reasonable to assume that binding of anions by γ-globulin would increase the extent of binding of protons at constant *p*H[26] and that the difference in the binding of cacodylate is a minor effect.[27] It will be further assumed that binding of the common ion, sodium, is the same in both buffers and that binding of cacodylic acid does not affect the valence of the protein. Thus, the absolute value of Δz would seem to represent a lower limit of the difference in binding of perchlorate and chloride, perchlorate being the more strongly bound. (It is possible, of course, that chloride is not bound by the protein, in which case Δz would correspond to a lower limit of the number of perchlorate ions bound.) Given this interpretation of Δz, the difference in the extent of binding of perchlorate and chloride by γ-globulin is about the same as in the case of isoionic human serum albumin at the same electrolyte concentration, and the minimum number of perchlorate ions that might be bound by γ-globulin is approximately one-half the number bound by serum albumin.[26] Reasoning in the above manner, one is led to conclude that the extent of binding[19] of chloride, bromide, nitrate and perchlorate by γ-globulin at *p*H 6.5 and 7.0 increases in the mentioned order. At *p*H 6 the protein has the same electrophoretic mobility in nitrate- and bromide-rich buffers.

Even though the changes in valence of γ-globulin produced by changes in buffer composition are about the same as those found by Longsworth and Jacobsen for bovine serum albumin as a result of binding of certain small ions, the resultant changes in the isoelectric *p*H values in the case of γ-globulin are considerably greater than in the case of serum albumin. This is probably to be interpreted in terms of a difference in the buffering capacities of the two proteins in the region of their respective isoelectric points. Comparison of our results with those of Longsworth and Jacobsen shows that in the case of γ-globulin the slope of the *p*H–mobility curve in the neighborhood of the isoelectric *p*H value is about one-half that of serum albumin. Thus larger *p*H shifts are required in the first case in order to compensate for changes in net charge on the protein resulting from changes in ion binding.

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(22) E. Fredericq and H. Neurath, THIS JOURNAL, **72**, 2684 (1950).

(23) G. Weber, *Biochem. J.*, **51**, 155 (1952); *Disc. Faraday Soc.*, **13**, 33 (1953).

(24) D. C. Henry, *Proc. Roy. Soc. (London)*, **A133**, 106 (1931).

(25) L. G. Longsworth and C. F. Jacobsen, *J. Phys. Colloid. Chem.*, **53**, 126 (1949).

(26) G. Scatchard and E. S. Black, *ibid.*, **53**, 88 (1949).

(27) Dr. Leonard Lerman, personal communication, has experimental evidence indicating that cacodylate is bound much less than chloride and perchlorate by methylated serum albumin.