Attempted Nitration of II without Cleavage of *t*-Butyl Group.—To 1.03 g. (0.005 mole) of I was added a solution of 0.315 g. (0.005 mole) of concentrated nitric acid in 2 ml. of glacial acetic acid, the temperature being kept below 0°. After several minutes, the mixture was poured onto cracked ice. The reddish-brown oil was washed by decantation with water and sodium bicarbonate, then dissolved in 95% ethanol. On dilution with water, crystals separated which, on recrystallization from aqueous ethanol, gave dark red needles, m.p. 245–247°.

Anal.[18] Calcd. for $C_{28}H_{40}O_2$: C, 82.2; H, 9.85. Found: C, 82.5, 82.7; H, 9.69, 9.60.

This product is probably 3,3′,5,5′-tetra-*t*-butyldiphenoquinone.

Rearrangement of II.—The warm solution of 5 drops of 98% sulfuric acid in 1 ml. of II was further heated for several minutes, then poured into cold water. The organic product was taken up in petroleum ether, and the latter washed with 10% aqueous alkali to extract any rearranged product. Acidification, followed by extraction with ligroin and evaporation of the solvent yielded crystals which, recrystallized from ligroin, melted 55–55.5°; mixed with an authentic sample of 2,4-di-*t*-butylphenol, 55–56°.

(18) Performed by Clark Micro Analytical Laboratories, Urbana, Illinois.

The absorption spectra were determined with a Beckman spectrophotometer (model DU) using 1-cm. quartz cells. The cyclohexane solvent (for the phenols) was freed of benzene by passage through silica gel, followed by fractionation. Absolute alcohol was the solvent for the quinone. The 2,6-di-*t*-butyl-4-methylphenol was obtained through the generosity of the Koppers Company, Pittsburgh, Penna., and was purified by distillation (146° at 20 mm.) and recrystallization from ligroin, m.p. 69–70°.

Relative Rates of Alkylation.—The apparatus consisted of a 125-ml. erlenmeyer flask equipped with a nitrogen bubbler and a condenser, the outlet of which was connected to small absorption tubes containing sodium carbonate. The flask was immersed in a constant temperature bath. The mixture of *t*-butyl chloride and substituted phenol was allowed to react, with a slow stream of dry nitrogen bubbling through the mixture. At the end of the time interval, the sodium carbonate tube was removed, its contents dissolved in distilled water, and an aliquot was acidified with nitric acid and analyzed for chloride by the Volhard method.

When nitrobenzene was used as the solvent, a blank experiment (in which the phenol was omitted) showed no dehydrohalogenation of the *t*-butyl chloride under the conditions of the experiment.

The results of these experiments are summarized in Table I.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

The Binding of Organic Ions by Proteins. Optical Evidence of Coöperative Interactions with Hydrogen Ions

BY IRVING M. KLOTZ AND JEAN M. URQUHART

Addition of sodium dodecyl sulfate to a buffered solution of iodinated human serum albumin or of iodinated bovine serum albumin produces a decrease in optical density of the protein in the region of 313 mμ. This drop in absorption demonstrates the uptake of hydrogen ions by the protein concurrently with the binding of anions. The magnitude of the effect is smaller than might be expected from simple electrostatic considerations.

The effect of bound anions on the acid-titration curves of proteins was first emphasized by Steinhardt[1] who estimated in addition the affinity of the protein for various anions. Changes in *p*H of isoionic albumin accompanying its interactions with dodecyl sulfate anions were also demonstrated by Putnam and Neurath.[2] More recently Scatchard and Black[3] have examined the uptake of hydrogen ions by isoionic albumin in the presence of a variety of anions and have used the observed shifts in *p*H to compute the number of bound anions. Similarly Longsworth and Jacobsen[4] have shown from their electrophoretic studies that anion binding may be accompanied by the uptake of protons.

From a complementary point of view, the electrostatic effect of bound hydrogen ions upon the affinity of proteins for anions has been considered in some detail by Scatchard, Scheinberg and Armstrong.[5] Experimentally, these investigators[5] have shown a marked increase in binding of chloride ion by cationic albumin as compared to anionic albumin molecules. Similar results have been reported[6] in studies with methyl orange at *p*H's acid to the isoelectric point of serum albumin. The increased binding of anions by positively-charged albumin can be accounted for quantitatively by electrostatic theory.[5]

In the present paper, changes in the optical properties of an iodinated serum albumin have been used to demonstrate proton uptake by the protein molecule (in buffered solutions) concurrently with the binding of anions. Quantitative estimates of the extent of combination of the protein with hydrogen ions, however, are smaller in magnitude than might be anticipated from calculations based on simple electrostatic considerations.

Experimental

Three iodinated albumins were used in these investigations, two of these being derivatives of human albumin and one of bovine albumin.

The derivatives of human origin were obtained through the kindness of Dr. W. L. Hughes, Jr., and Dr. R. Straessle[7] of the Department of Physical Chemistry of the Harvard Medical School. One sample ("50% iodinated") contained sufficient iodine to convert one-half of the (eighteen) tyrosine residues in human albumin to diiodotyrosine. The second sample ("100% iodinated") contained enough iodine to saturate all eighteen tyrosine groups, but chemical evidence indicates that only about thirteen were covered.[7]

An estimate of the number of diiodotyrosine groups in these protein derivatives can be made also from their optical absorption. The optical density of a 0.100% solution of the 100% iodinated material at 313 mμ was approximately 0.83 at *p*H 11.1. From the data of Crammer[8] and Herriott[9] on diiodotyrosine, one may estimate a value of

(1) J. Steinhardt, *Ann. N. Y. Acad. Sci.*, **41**, 287 (1941).

(2) F. W. Putnam and H. Neurath, THIS JOURNAL, **66**, 692 (1944).

(3) G. Scatchard and E. S. Black, *J. Phys. Colloid Chem.*, **53**, 88 (1949).

(4) L. G. Longsworth and C. F. Jacobsen, *ibid.*, **53**, 126 (1949).

(5) G. Scatchard, I. H. Scheinberg and S. H. Armstrong, Jr., THIS JOURNAL, **72**, 535, 540 (1950).

(6) I. M. Klotz and J. M. Urquhart, *ibid.*, **71**, 1597 (1949).

(7) W. L. Hughes, Jr. and R. Straessle, *ibid.*, **72**, 452 (1950).

(8) J. L. Crammer and A. Neuberger, *Biochem. J.*, **37**, 302 (1943).

(9) R. M. Herriott, *J. Gen. Physiol.*, **31**, 19 (1947).

about 5500 for the molecular extinction coefficient at 313 mμ. If the molecular extinction coefficient of the amino acid is about the same in the protein as in the free state, an optical density of 0.83 corresponds to 11 diiodotyrosine residues per molecule of human albumin. An analogous calculation with the data for the 50% iodinated material gives a value of 6 residues.

The sample of iodinated bovine serum albumin was obtained through the courtesy of Dr. G. R. Greenberg of the Department of Biochemistry of the School of Medicine of Western Reserve University. The optical absorption of this protein indicated the presence of nearly 6 residues of diiodotyrosine per albumin molecule.

The sodium dodecyl sulfate was a specially purified sample generously supplied by E. I. du Pont de Nemours and Co. Urea and urethan were Eastman Kodak Co. "highest purity" grade. Buffers were prepared from reagent grade acetate, phosphate and borate salts and reagent grade acetic acid and sodium hydroxide. The acetate buffer was 0.2 *M*, the phosphate 0.1 *M* and the borate 0.05 *M* in concentration.

In experiments with albumin and dodecyl sulfate, separate, buffered solutions of about 0.1% concentration, accurately known, were prepared for each substance. One sample of albumin was diluted with an equal quantity of buffer solution, and another was diluted with an equal quantity of the dodecyl sulfate solution. The spectrum of each solution was then examined. The dodecyl sulfate shows no significant absorption of ultraviolet light in the region covered.

With urea and urethan, however, this procedure was not convenient, because large quantities of these substances are necessary to prepare 6 and 1 *M* solutions, respectively. The required quantity of urea, or urethan, was added, therefore, to the initial buffer solution, and the albumin was dissolved in this mixed solvent. The spectrum of the protein was then compared with that of an equal weight of albumin dissolved in an equivalent volume of buffer alone.

Spectra were taken in 1-cm. silica cells with the Beckman quartz spectrophotometer, model DU, at 24 ± 2°.

Results

Effect of *p*H on Spectra of Iodoalbumin.—Ultraviolet absorption spectra of iodinated human serum albumins at *p*H 10.75 have been described by Hughes and Straessle.[7] In connection with the present investigation, corresponding spectra were obtained in buffered solutions over a *p*H range from 4 to 11. Optical densities, $E^{1\%}_{1\,cm.}$ at 313 mμ for the "100% iodinated" sample, as a function of *p*H, are illustrated in Fig. 1.

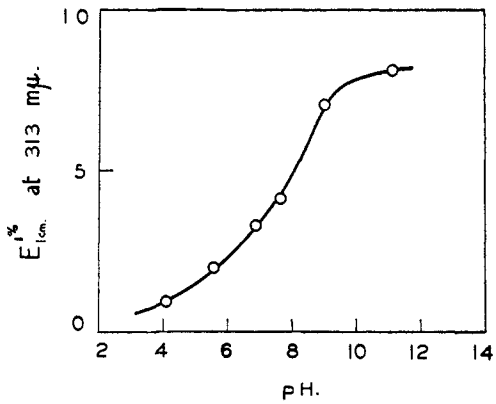

Fig. 1.—Optical densities of iodinated human serum albumin as a function of *p*H.

The dependence of absorption on *p*H resembles a titration curve very closely. The midpoint of the curve seems to be near *p*H 7.6. The asymmetric form of this titration curve, in contrast to that which one observes normally with simple acids, can be attributed to electrostatic interactions in the protein molecule. It seems reasonable to assume, therefore, that the diiodotyrosine residues in iodinated human serum albumin behave as acids with an average *pK* near 7.6.

The corresponding free amino acid has a *pK* of 6.48 according to Dalton, Kirk and Schmidt,[10] or of 6.42 according to Crammer and Neuberger.[8] The deviation between the value for the free amino acid and for the residue in the protein is in the direction to be expected from electrostatic interactions within the protein molecule. A similar deviation has been observed for tyrosine,[11] in which case the average *pK* is 11.7 for the residues in serum albumin as contrasted to 10.1 for the *pK* of the free amino acid. The large difference in *pK*'s for tyrosine, as compared to diiodotyrosine, may be attributed to the fact that the protein in the former case is further from its isoelectric point, and hence electrostatic interactions affect the ionization constant more strongly.

The uptake of a proton by the phenoxide ion of the diiodotyrosine residue thus is accompanied by a decrease in the optical density of the protein near 313 mμ. Iodinated albumin, therefore, is ideally suited for an examination of the effect of binding of organic anions and molecules on the proton equilibria of the protein basic to its isoelectric point.

Interactions with Dodecyl Sulfate.—The effect of sodium dodecyl sulfate on these equilibria was examined first. This substance is especially convenient for the present purpose since it does not absorb radiation in the region of 300 mμ, and does not affect significantly the *p*H of a buffer, yet is bound very avidly by serum albumin.[2,12,13]

The effect of sodium dodecyl sulfate on the proton equilibria of the "100% iodinated" human albumin at *p*H 7.6 is illustrated in Fig. 2. The addition of the anion to a concentration of 1.73×10^{-3} *M* changes the optical absorption of the (7×10^{-6} *M*)

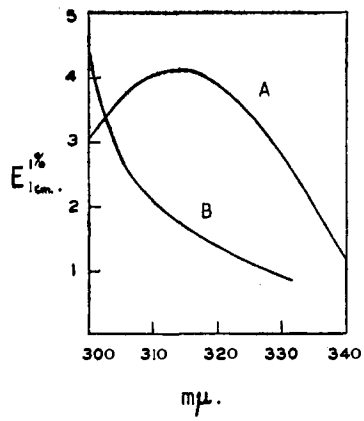

Fig. 2.—Effect of sodium dodecyl sulfate (1.73×10^{-3} *M*) on the optical density of "100% iodinated" human albumin (6.6×10^{-6} *M*) in phosphate buffer at *p*H 7.6. Curve A is that of protein alone, curve B that of protein with dodecyl sulfate.

(10) J. B. Dalton, D. L. Kirk and C. L. A. Schmidt, *J. Biol. Chem.*, **88**, 589 (1930).

(11) C. Tanford, THIS JOURNAL, **72**, 441 (1950).

(12) F. Karush and M. Sonenberg, *ibid.*, **71**, 1369 (1949).

(13) I. M. Klotz, H. Triwush and F. M. Walker, *ibid.*, **70**, 2935 (1948).

protein at *p*H 7.6 to a curve which is characteristic of *p*H 5.4. The titration curves of Tanford[11] indicate that such a *p*H change would be accompanied by an uptake of about 15 protons.

Similar effects have been observed with the "50% iodinated" human albumin at several *p*H's from 5.7 to 9.2 (Fig. 3). In each case, the addition of sodium dodecyl sulfate to a concentration of 1.7×10^{-3} *M* lowered the optical density of the iodoprotein and hence was accompanied by an uptake of protons by the albumin. The drop in optical density at *p*H 9.2 corresponds to a decrease which would be observed if the *p*H were changed to 7.7; the change in the solution of *p*H 7.6 corresponds to that for a *p*H drop to 5.5; the lowering in the solution of *p*H 5.6 produces a spectrum characteristic of *p*H 4.2. The titration curves of Tanford[11] indicate that these *p*H changes correspond to an uptake of 11, 15 and 26 protons, respectively.

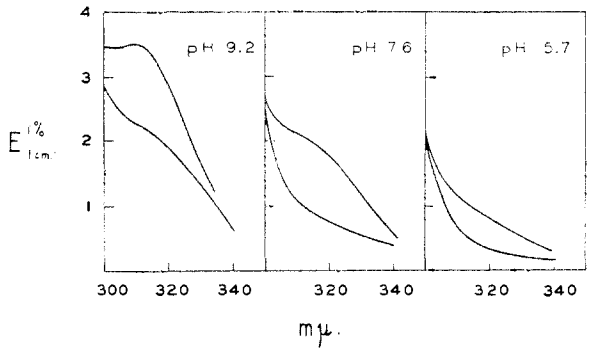

Fig. 3.—Effect of sodium dodecyl sulfate (1.7×10^{-3} *M*) on the optical density of "50% iodinated" human albumin (7.0×10^{-6} *M*). Borate buffer was used for *p*H 9.2, phosphate for *p*H 7.6 and *p*H 5.7.

The behavior of the iodinated bovine serum albumin is illustrated in Fig. 4. The depressions in optical density produced by the addition of sodium dodecyl sulfate parallel those observed with the corresponding human albumin containing 6 iodotyrosines. Evidently the changes in proton equilibria are also of comparable magnitude in both proteins.

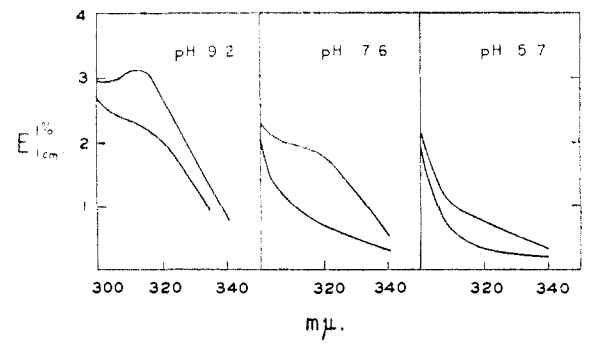

Fig. 4.—Effect of sodium dodecyl sulfate (1.7×10^{-3} *M*) on the optical density of iodinated bovine albumin (7.0×10^{-6} *M*). Buffers same as those with human albumin.

If the data summarized in Figs. 3 and 4, respectively, are plotted in terms of absorption at any fixed wave length *versus* *p*H, curves similar to the left-hand portions of Fig. 1 are obtained. In each case, *i.e.*, for human and bovine albumin, respectively, the optical titration curve in the presence of dodecyl sulfate is displaced by somewhat less than 2 *p*H units toward higher *p*H's, but is essentially parallel to the curve obtained in the absence of these anions.

The curves of Fig. 3 or of Fig. 4 also indicate that the dodecyl sulfate does not affect the absorption of iodoalbumin in any manner other than through its influence on the proton equilibria of the protein. Thus the lower curve at *p*H 9.2, obtained in the presence of alkyl sulfate, is similar in shape to the upper curve at *p*H 7.6, observed in the absence of dodecyl sulfate. Similar conclusions can be reached from a comparison of the curve at *p*H 7.6 in the presence of dodecyl sulfate with that at *p*H 5.7 in the absence of this anion.

It was not possible to examine the effect of dodecyl sulfate on the optical properties of albumin at strongly acid *p*H's since a precipitate is formed at *p*H's below the isoelectric point of the protein.[2] Nevertheless, it is obvious from Figs. 3 and 4 that even at *p*H 5.7 the protein in the presence of alkyl sulfate shows the depressed absorption characteristic of pure iodoalbumin below its isoelectric *p*H. No attempt was made to observe the influence of alkyl sulfate on the absorption of iodoalbumin in a state such that its iodotyrosine residues are completely in the phenoxide form, since a *p*H of about 13 would be required and substantial damage might be inflicted on the protein in such a basic solution.

The concentration of dodecyl sulfate used in the experiments described so far produces very striking effects on the absorption of albumin. Since some objection might be raised, however, on the grounds that 1.7×10^{-3} *M* is above the critical concentration[14] of dodecyl sulfate, an experiment (at *p*H 7.6) was carried out also at a concentration of 4.4×10^{-4} *M*, well below the critical concentration. The results obtained, with bovine iodoalbumin, were similar to those described in Fig. 4, except that the lowering in absorption produced by dodecyl sulfate was less at this lesser concentration. The absorption curve (at *p*H 7.6) of the iodoalbumin in the presence of 4.4×10^{-4} *M* dodecyl sulfate was characteristic of the pure protein at *p*H 6.4. This change in *p*H corresponds to an uptake of 9 protons by the iodoprotein. In these dilute dodecyl sulfate solutions, almost all of the anion is bound by the protein.[15] It is apparent, therefore, that the uptake of 9 protons occurs concurrently with binding of around 50 dodecyl sulfate anions.

Interactions with Urea or Urethan.—In marked contrast to dodecyl sulfate are the effects produced by either 6 *M* urea or 1 *M* urethan. Previous investigations by dye-displacement techniques[13] have demonstrated extensive binding of urea and of urethan by bovine serum albumin. Optical studies with the iodinated bovine albumin show very little effect for either urea or urethan (Table I). A small increase in absorption occurs in the presence of either of these substances, in contrast to the marked decreases produced by dodecyl sulfate.

(14) M. L. Corrin and W. D. Harkins, THIS JOURNAL, **69**, 683 (1947).
(15) F. Karush, private communication.

Since urea or urethan is present in such large concentrations, a slight change in absorption is of little significance for it may be due to a pronounced change in the properties of the aqueous solvent.

TABLE I

EFFECT OF UREA AND OF URETHAN ON OPTICAL DENSITY OF IODINATED BOVINE ALBUMIN

Wave length, mμ	Optical density[a] Protein alone	Protein in 6 *M* urea	Protein in 1 *M* urethan
300	0.253	0.261	0.273
310	.238	.250	.246
320	.214	.225	.218
330	.141	.150	.142
340	.061	.066	.060

[a] All solutions were in a phosphate buffer, which by itself had a *p*H of 7.8.

Discussion

The difference in behavior of urea and dodecyl sulfate indicates clearly that the effects of the latter on the proton equilibria of iodoalbumin are due to its electrostatic influence rather than to a general modification of the protein molecule. Furthermore, the absence of any appreciable effect on the optical properties of iodoalbumin in the region of 313 mμ when either urea or urethan is added confirms the assumption that changes in absorption in this wave length region are due to the loss or gain of hydrogen ions by the protein. It is of interest, therefore, to see to what extent electrostatic theory can account quantitatively for the changes observed.

In the absence of dodecyl sulfate anions, the proton equilibrium of an iodotyrosine group of albumin may be represented by the equation

$$P + H^+ = PH^+ \qquad (1)$$

whereas in the presence of dodecyl sulfate

$$PA_n + H^+ = PA_n H^+ \qquad (2)$$

In equation (2) A_n represents the bound anions. If the equilibrium constant for the association in equation (1) is represented by k and that for the process in (2) by k', then the ratio k'/k refers to the process

$$PA_n + PH^+ = PA_nH^+ + P \qquad (3)$$

and ΔF° for reaction (3) is given by

$$\Delta F^\circ = -RT \ln (k'/k) \qquad (4)$$

It is possible to calculated k'/k, and hence ΔF°, from the experimentally-determined optical densities. From equation (3) it is apparent that

$$\frac{k'}{k} = \frac{(P)(PA_nH^+)}{(PH^+)(PA_n)} \qquad (5)$$

Knowing the optical densities of the iodotyrosine group in acid and basic solutions, we can determine the value of the quotient $(P)/(PH^+)$ from the optical density in the buffer without dodecyl sulfate, and that of $(PA_nH^+)/(PA_n)$ from the optical density in the same buffer with the known quantity of added dodecyl sulfate. These values are listed in Table II, together with the subsequently computed quantities, k'/k and ΔF°.

The value of ΔF° for reaction (3) to be expected on the basis of electrostatic effects can be determined by a procedure analogous to that used previously[16] in an analysis of electrostatic effects on protein binding. If one assumes that the difference between k' and k is due entirely to the additional charge of the bound anions in PA_n, then

$$\Delta F^\circ = -2n \frac{Ne^2}{2D}\left[\frac{1}{b} - \frac{\kappa}{1+\kappa a}\right] \qquad (6)$$

if each A ion has a charge of -1. In this equation N represents Avogadro's number, e the electronic charge, D the dielectric constant of the medium, b the radius of the protein molecule, a the distance of closest approach of a small ion to the protein and κ the well-known factor in the Debye–Hückel theory which includes the ionic strength.

Given the known ionic strength of the buffer solutions one can calculate readily the factor $\frac{Ne^2}{2D}\left(\frac{1}{b} - \frac{\kappa}{1+\kappa a}\right)$. The values obtained are listed in Table II. This electrostatic factor combined with the experimentally-determined ΔF° permits one to compute n. These values have been placed in the last column of Table II.

TABLE II

CALCULATION OF BOUND DODECYL SULFATE ANIONS FROM ELECTROSTATIC THEORY

		$\frac{(PH^+)}{(P)}$	$\frac{(PA_nH^+)}{(PA_n)}$	$\frac{k'}{k}$	$-\Delta F^\circ = RT \ln (k'/k)$ cal./mole	μ	$\frac{Ne^2}{2D}\left(\frac{1}{b} - \frac{\kappa}{1+\kappa a}\right)$ cal./mole	n
Iodo-human albumin 13 iodotyrosines, *p*H 7.6		1.02	3.61	3.5	740	0.260	16.0	23
Iodo-human albu-	*p*H 9.2	0.175	0.860	4.9	940	.150	18.4	26
min 6 iodotyro-	*p*H 7.6	0.966	3.22	3.3	710	.260	16.0	22
sine residues	*p*H 5.7	2.9	6.7	2.3	490	.110	20.2	12
Iodo-bovine albumin	*p*H 9.2	0.194	0.635	3.3	710	.150	18.4	19
6 iodotyrosine	*p*H 7.6	0.948	2.84	3.0	650	.260	16.0	20
residues	*p*H 5.7	3.1	6.7	2.2	470	.110	20.2	12
	*p*H 7.6[a]	0.993	1.88	1.9	380	.260	16.0	12

[a] In this experiment the concentration of sodium dodecyl sulfate was 4.4×10^{-4} *M*; in all the other experiments it was 1.7×10^{-3} *M*.

It is apparent that in solutions of 1.7×10^{-3} *M* dodecyl sulfate, computed n values are near 20, and in the 4.4×10^{-4} *M* solution, near 12. These computed results are far below the number of dodecyl sulfate anions which are actually bound by albumin. The experiments of Putnam and Neurath,[17] carried out under conditions similar to those in the 1.7×10^{-3} *M* solutions of the present paper, indicate that all the available sites on the albumin molecule have combined with dodecyl sulfate anions. The number of bound anions thus

(16) I. M. Klotz, F. M. Walker and R. B. Pivan, THIS JOURNAL, **68**, 1486 (1946).

(17) F. W. Putnam and H. Neurath, *J. Biol. Chem.*, **159**, 195 (1945).

must be in the neighborhood of 100 per albumin molecule. Similarly in the solution of 4.4×10^{-4} dodecyl sulfate, the number of bound anions must be somewhat over 50.

Strictly speaking some correction should be made for any displacement of buffer anions by dodecyl sulfate. It becomes apparent upon detailed consideration, however, that such a correction must be small. Comparative studies of various buffer ions[18] indicate that acetate, phosphate and glycinate are bound less strongly than chloride. Other unpublished data have shown also that borate and glycinate are comparable in their binding ability. Since at concentrations near 0.1 *M* only about six chloride ions are bound by albumin,[5] it seems reasonable to assume that no more than six ions of any one of the buffers used in the present study are bound by albumin. Even if six buffer ions are completely displaced by added dodecyl sulfate, the change in charge is much too small to account for the difference between the calculated and experimental values described above.

In addition to displacing buffer anions from the protein, the bound dodecyl sulfate causes an uptake of protons by albumin. It seems reasonable to assume that the changes in proton equilibria which are obvious for iodinated tyrosine residues also occur, under comparable electrostatic conditions, in other basic and acidic side-chains in the albumin molecule. The number of bound protons may then be estimated, as has been pointed out above, as 11, 15 and 26 at *p*H's 9.2, 7.6 and 5.7, respectively (when the dodecyl sulfate concentration is 1.7×10^{-3} *M*). These numbers, however, even if added to the number of displaced buffer ions do not account for the discrepancy of approximately 80 between the value of 20 calculated with the aid of equation (6) and that of over 100 indicated from experimental measurements. Thus it becomes apparent that the observed electrostatic effect of the bound dodecyl sulfate ions is less than would be expected from theoretical considerations.

A similar discrepancy seems to be present on the acid side of the isoelectric point of serum albumin as judged from the titration data of Tanford.[11] The observed electrostatic effect of added protons in the acid region is less than would be expected on theoretical grounds if only protons are bound to the protein. To bring observations and theory into agreement Tanford found it necessary to assume that a large number of chloride ions are bound by albumin. Direct observations[5] indicate, however, that the number of chlorides actually bound is about half the number suggested from titration data.

The discrepancy between electrostatic theory and experiment described in the present paper might be attributed at first glance to an effect of dodecyl sulfate on the optical absorption in addition to that due merely to electrostatic factors. Such an explanation seems unlikely, however, since 1 *M* urethan and 6 *M* urea produce only minor changes in the optical density. Perhaps instead we have reached a point where the electrostatic interaction term of equation (6) cannot be applied in so simple a manner. In the present experiments, the number of bound anions is much larger than in any quantitative equilibrium studies described previously.[5,6,12,16] With a large number of bound anions the charge density of the protein becomes great. Under such conditions other cations in the buffer solution, such as Na^+, may be trapped within the protein molecule. It has been demonstrated in many ways that synthetic polymeric electrolytes are capable of immobilizing small ions of opposite charge.[19,20,21] An appreciable uptake of Na^+ ions by the albumin–dodecyl sulfate complex could account for the apparently reduced electrostatic effect of the bound anions.

Acknowledgment.—This investigation was greatly facilitated by grants from the Rockefeller Foundation and from the Office of Naval Research (Project No. NR121-054). We are also indebted to Miss Janet Ayers for several of the spectra described.

EVANSTON, ILL. RECEIVED OCTOBER 12, 1950

(18) I. M. Klotz and J. M. Urquhart, *J. Phys. Colloid Chem.*, **53**, 100 (1949).

(19) W. Kern, *Makromol. Chem.*, **2**, 279 (1948).

(20) G. I. Cathers and R. M. Fuoss, *J. Polymer Sci.*, **4**, 121 (1949).

(21) J. R. Huizenga, P. F. Grieger and F. T. Wall, THIS JOURNAL **72**, 2636, 4228 (1950).