THE MOLECULAR MECHANISM OF COLLOIDAL BEHAVIOR. III. THE CHEMICAL NATURE OF THE ADSORPTION OF ACIDS AND ALKALIES BY THE PROTEIN MOLECULE.

By Russell S. Bracewell.

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In two previous articles[1] the behavior of blood fibrin in acids and alkalies has been studied, and a correlation between the swelling of the fibrin and the adsorption of acid and alkali shown. The purpose of the present article is to discuss the nature of this adsorption process.[2]

The older theories of adsorption assumed that the phenomenon was produced by the action of "physical forces" having somewhat the general character of gravitational attraction. Our newer ideas of adsorption,[3] however, consider the attraction to be more chemical in its nature, arising from stray fields of "chemical force" around atoms whose chemical affinity has not been completely satisfied. On the basis of these newer ideas, we should predict, from the amphoteric chemical nature of the protein molecule, that it would adsorb either hydrogen ion or hydroxide ion, according as to whether the solution is acid or alkaline, and these are found to be the experimental facts, as was shown, for example, in the two previous articles already mentioned. The work cited, moreover, showed not only an adsorption of both acids and alkalies, but also, at least with the strong acids and alkalies, that there is a fairly definite maximum number of equivalents of acid or base adsorbed by the fibrin, which is not markedly increased even by greatly increasing the concentration of the supernatant solution. This affords additional support to the idea that the adsorption process is dependent upon chemical factors and indicates that there may be a limited number of positions on the protein molecule where hydrogen ions or hydroxide ions tend to attach themselves, and that after these positions have been saturated, the protein molecule has become electrically charged to such a degree that further "indiscriminate" adsorption does not take place.

As a matter of fact it appears that the amount of acid "neutralized" by a considerable number of proteins is determined by the number of free amino groups in the molecule. This conclusion differs from that reached by Robertson[4] who, however, based his figures for the number

[1] Tolman and Stearn, This Journal, 40, 264 (1918); Tolman and Bracewell, *Ibid.*, 41, 1503 (1919).

[2] Since writing this article the author has noted the statement by Harkins, Davies, and Clark (This Journal, 39, 541 (1917)) that the necessary condition for an adsorption of the kind discussed in the present paper is probably determined by the polar groups in the molecule.

[3] Langmuir, This Journal, 40, 1361 (1918).

[4] "The Physical Chemistry of the Proteins," Longmans Green & Co., 1918, p. 20.

of free amino groups solely upon the criterion that such groups give nitrogen with nitrous acid, whereas the work of Kossel and Cameron[1] indicates that in the case of arginine the amino group in the guanidine nucleus must be regarded as free in spite of the fact that it does not react with nitrous acid. As to the neutralizing power of proteins for alkalies, no definite relation has been found between the amount of base fixed and the structure of the protein molecule. This is in agreement with the conclusions of Osborne, Leavenworth and Brautlecht[2] and of Osborne and Van Slyke[3] that, even in the case of the dicarboxylic acids, glutamic and aspartic, there are no free COOH groups but that these are bound by the free ammonia which is always obtained on the hydrolysis of proteins containing these acids. We may conclude that the adsorption of hydroxide ion by proteins will be of a less specific character.

Experimental Data.

The following table compares data on the composition of the more common proteins whose amino acid content has been determined by the Van Slyke group method[4] with a fair degree of accuracy, with data on the maximum adsorption of acid by these proteins.

TABLE I.

Protein.	Total N. %	% total N as lysine.	% total N as arginine.	Free NH_2 groups calculated per g. of protein. Equivalents $\times 10^5$.	Acid adsorbed per g. of protein. Equiv. $\times 10^5$.
Fibrin	16.91	11.51	13.86	108	96
Gelatin	17.97	6.32	14.70	85	70
Casein	17.78	10.30	7.41	79	60
Gliaden	17.50	0.75	5.71	23	28
Edestin	18.65	3.86	27.05	115	127

The figures for total nitrogen in these proteins are taken from the work of Van Slyke,[5] for the percentages of this nitrogen combined as lysine and arginine in casein from Van Slyke,[6] and for the other proteins from the work of Matthews.[7] The adsorption data on fibrin, gelatin, gliadin and casein were obtained by titrimetric methods, with phenolphthalein as indicator, using hydrochloric acid over the protein as described in a previous article.[8] The fibrin was also therein described. The gelatin was the commercial high grade slab gelatin. The casein and gliadin were prepared by graduate students in the Laboratory of Physiological

[1] *Z. physiol. Chem.*, **76**, 457 (1912).
[2] *Am. J. Physiol.*, **23**, 180 (1908–9).
[3] *J. Biol. Chem.*, **22**, 259 (1915).
[4] *Ibid.*, **10**, 15 (1911–12).
[5] *Loc. cit.*
[6] *J. Biol. Chem.*, **16**, 538 (1915).
[7] "Physiological Chemistry," William Wood & Co., **1916**.
[8] Tolman-Bracewell, *Loc. cit.*

Chemistry of the University of Illinois. The acid adsorption data for edestin are taken from Robertson.[1] The adsorption figures, in view of the uncertainty of reproducing colloidal phenomena and of the method of measurement, which involves determining a small change in concentration by difference, are probably subject to an error of something less than 10%. Data by the Van Slyke group method indicates that this method gives results with an error of about 5%.

In these five proteins, lysine and arginine are the only acids with free amino groups, and hence we need only consider these two acids in calculating the "neutralizing power" of these proteins for acid. In the case of lysine, $H_2NCH_2 — CH_2 — CH_2 — CH_2 — CHNH_2 — COOH$, $^1/_2$ of the nitrogen reacts with nitrous acid and hence is assumed to be combined in a free amino group, the other amino group being combined with the carboxyl of another molecule of acid to form the protein chain. In the case of arginine,

$$H_2N — \underset{\underset{NH}{\|}}{C} — NH — CH_2 — CH_2 — CH_2 — CHNH_2 — COOH,$$

none of the nitrogen reacts with nitrous acid and yet from the structure of the molecule, we may conclude, in agreement with the views of Kossel and Cameron,[2] that the molecule contains one free amino group not bound in the peptide linkage. Proceeding then on the assumption that $^1/_2$ of the lysine nitrogen and $^1/_4$ of the arginine nitrogen is combined in a free amino group, the number of free amino groups per g. of protein has been calculated and is given in the fifth column of Table I. A comparison of these figures with the number of equivalents of acid adsorbed shows reasonable agreement, from which we may conclude that the acid-adsorbing power of proteins is mainly due to the free amino groups present. Similar data were obtained for serum globulin, excelsin, amandin, and legumin, which show the same parallelism, though the divergences are slightly greater, owing probably to the circumstances that the compositions used in the calculations are derived from old methods of analysis and therefore are not well established.

In further support of our theory it may be pointed out that Goto[2] showed that clupein, which contains no lysine, binds approximately one equivalent of sulfuric acid for each molecule of arginine. As another example, Basil and Matula[3] and Pauli and Hirschfeld[3] have given the values 1.65×10^{-2} and 0.85×10^{-2} for the acid adsorbed from 0.05 N hydrochloric acid solution by normal and deaminized gelatin, respectively; the value 1.65×10^{-2} represents the adsorption by the free amino groups in both lysine and arginine, whereas the value 0.85×10^{-2} represents the adsorption by the free amino group in arginine alone since the latter is

[1] *Loc. cit.*, p. 103.

[2] *Z. physiol. Chem.*, **37**, 114 (1902).

[3] *Loc. cit.*

not deaminized by nitrous acid, and consequently the difference 0.8×10^{-2} represents the combining capacity of the lysine. If we check this against the known composition of gelatin we find that the ratio of $^1/_4$ the arginine nitrogen to $^1/_2$ the lysine nitrogen is 1.2:1, whereas the ratio of the above adsorption values is unity.

Robertson[1] states that he formerly held the view here presented, that the acid-combining power of a protein is determined by the number of free amino groups, but that in view of more recent work he must conclude that some factor other than the number of free amino groups is responsible for the acid neutralizing power possessed in such a high degree by some proteins. It is believed, however, by the writer, that Robertson has failed to consider the possibility of free amino groups in arginine which do not react with nitrous acid. His statement that 100 equivalents of sturin neutralize no less than 24 equivalents of acid, does not seem unreasonable, when we consider that $^1/_2$ the lysine nitrogen plus $^1/_4$ the arginine nitrogen gives us a figure of 20 equivalents. In considering salmin he concluded that since it gives no nitrogen with nitrous acid, this protein contains no free amino groups; from Kossel and Cameron's work[2] it appears, however, that the amino groups on the guanidine end of the arginine molecule might be free and yet not react with nitrous acid to give nitrogen.

Considering now the positions on the protein molecule most likely to have affinity for the hydroxyl ions we might look to the dicarboxylic acids, but since the COOH groups of glutamic and aspartic acids which are not bound up in the peptide linkage are combined in an amide group with ammonia, it is likely that the affinity at these points would be small. Calculations bear this out in that there is no definite relation between the base adsorbed and the dicarboxylic acid content of the proteins. The effect of dil. alkali in racemizing proteins is of interest in this connection. According to Dakin and Dudley's[3] theory the alkali is the factor in effecting the keto-enol transformation in the peptide linkage which renders the amino acids optically inactive. In such cases it is quite probable that one hydroxyl ion is held at each peptide junction where this transformation occurs. An attempt to correlate alkali adsorption experimental data with their work on racemization was unsuccessful, however, the data are meager and it may be that the method discussed may help in elucidating this question. In general it seems that the proteins having a high affinity for alkalies are high in the simple, straight chained amino acids, but attempts at further correlation failed.[4]

[1] *Loc. cit.*, p. 20.

[2] *Loc. cit.*

[3] *J. Biol. Chem.*, **15**, 271 (1913).

[4] Mr. Bracewell has been good enough to show me the material in his article. I am inclined to agree with him that the amount of acid adsorbed by proteins is de-

The work described in this article was performed in the chemical laboratory of the University of Illinois.

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[CONTRIBUTION FROM THE GEOPHYSICAL LABORATORY OF THE CARNEGIE INSTITUTION OF WASHINGTON.]

APPLICATION OF THE THERMIONIC AMPLIFIER TO CONDUCTIVITY MEASUREMENTS.[1]

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The use of the thermionic amplifier as an aid in making conductivity measurements is an outgrowth of the necessity for accurate work under restricted conditions. We have been confronted with the need of making resistance measurements on saturated solutions of electrolytes in cells whose volume is small and whose electrodes are limited in area to approximately 2 sq. mm. Under these conditions, to avoid heating effects during a measurement, and to minimize polarization at the electrodes, as far as possible, the current through the cell should be made as small as is compatible with a correct bridge setting; and the lower limit to the value of the current is determined by the sensitivity of the telephone.

Ordinarily the conductance cell is designed to meet the requirements of the telephone which is to be used with it. A good telephone has an audibility current of 10^{-8} or 10^{-9} amperes, audibility current being defined as the least current through the telephone which will produce an audible tone. For the majority of uses in connection with measuring conductivities this is sufficient sensitivity. If one is not confronted with limitations in the size and spacing of the cell electrodes, and can work in a quiet room, there is little difficulty in satisfactorily determining the minimum, for one can vary the current passing through the bridge until

termined by their content of the two diamino acids, lysine and arginine. It should not be concluded from this, however, that a protein containing no lysine or arginine would adsorb no acid at all. I believe that, owing to their amphoteric nature, all proteins would adsorb some acid, in the form of a double layer of hydrogen ion and acid ion; the electrostatic force set up by this double layer, however, would discourage further adsorption, and with increasing concentration of acid the adsorption would finally cease to increase. If now, lysine or arginine are present in the protein, the adsorbed acid will be mainly fixed by the strong forces produced by the free amino groups of these acids; after these forces are neutralized, further indiscriminate adsorption at places on the molecule having weaker forces will be prevented by the electrical charge which has been set up. On this basis we should expect the amount of acid adsorbed by ordinary proteins, containing lysine and arginine, to be determined mainly by the content of these diamino acids, as has been found by Mr. Bracewell.

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[1] Presented at the Buffalo meeting of the American Chemical Society, April 7–11, 1919.