ABSTRACTS OF COMMUNICATIONS.

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On the mode of union of certain proteins with acids and bases.

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Considerable experimental evidence has in recent years been brought forth which indicates that the union of proteins with acids and bases takes place by chemical combination.[1] It has been suggested by Robertson[2] that the -COHN- groups in the protein molecule are responsible for the acid- and base-combining capacity but this hypothesis has been recently questioned by ourselves.[3] Kossel and Cameron[4] pointed out many years ago that the acid-combining capacity of salmin is equal to the combining capacity of the guanidine groups in the arginine, and Bracewell[5] has carried this idea further by showing in a number of proteins a close agreement between the acid-combining capacity and the sum of the free amino nitrogen and the amino group of arginine which does not react with HNO_2. The recent work of Hitchcock[6] in which he determined the acid-combining capacity of gelatin and of deaminized gelatin affords an opportunity for checking this hypothesis and our calculations which are given in Table I show an excellent agreement between the calculated and the experimental results.

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[1] Loeb, J., *Proteins and the Theory of Colloidal Behavior*, New York, 1922.

[2] Robertson, T. B., *The Physical Chemistry of the Proteins*, p. 24.

[3] Greenberg, D. M., and Schmidt, C. L. A., PROC. SOC. EXP. BIOL. AND MED., 1923, xxi, 197.

[4] Kossel, A., and Cameron, A. T., *Z. physiol. Chem.*, 1912, lxxvi, 457.

[5] Bracewell, R. S., *J. Am. Chem. Soc.*, 1919, xli, 1511.

[6] Hitchcock, D. I., *J. Gen. Physiol.*, 1923, vi, 95.

TABLE I.

Combining Power of Gelatin for Acids.

Amino Acids	Per cent of total N	Mols N which combine with acid	Mols HCl which should be bound	Mols HCl which are bound (based on Hitchcock's data)
Arginine nitrogen[10]	14.7	47×10^{-5}	47×10^{-5}	45×10^{-5}
Lysine nitrogen[10]	6.3	41×10^{-5}	41×10^{-5}	44×10^{-5}

The discovery of β-hydroxy-glutamic acid in certain proteins by Dakin[7] and the more recent work by Harris[8] showing that tyrosine must be regarded as a dibasic acid has given us data which we have used in attempting to determine the seat of the base-combining power in the protein molecule. The early experiments of Osborne and his collaborators indicate that the second carboxyl group of the dibasic amino acids does not exist free in certain of the vegetable proteins but is combined as the acid amide. This view is probably not strictly true, especially if the content of β-hydroxy-glutamic acid is considered. In the instance of casein which is predominantly acid the discrepancy between the amide nitrogen and the content of dicarboxylic acids is considerable. The experiments of Osborne and Nolan[9] in which they found a direct relationship between the increase in base-combining power of certain proteins and the ammonia which was split off by mild hydrolysis strongly points to the dicarboxylic acids as the seat of the base-combining power of the protein molecule.

Accurate data upon which to base calculations are available only for several proteins and while our data (Table II) do not

[7] Dakin, H. D., *Biochem. J.*, 1918, xii, 290.

[8] Harris, L. J., *Proc. Roy. Soc.*, 1923, xcv, 440.

[9] Osborne, T. B., and Nolan, O. L., *J. Biol. Chem.*, 1920, xliii, 311.

[10] Dakin, H. D., *J. Biol. Chem.*, 1920, xliv, 499.

TABLE II.

Base-combining Power of Casein.

Amino Acids	Mol. Wt.	Per cent	Mols of NaOH which should be bound (per gm. of protein)
Glutamic[7]	147	21	143×10^{-5}
Aspartic[7]	133	3.8	26×10^{-5}
β-hydroxy-glutamic[7]	163	10.5	64×10^{-5}
Tyrosine[11]	181	5.8	32×10^{-5}
			Total 265×10^{-5}
Less amide nitrogen[11]	14	1.5	107×10^{-5}

Mols NaOH which should be bound, 158×10^{-5}.
Mols NaOH which one gm. of casein binds at pH 12, 160×10^{-5}.

Base-combining Power of Gelatin.

Amino Acids	Mol. Wt.	Per cent	Mols of NaOH which should be bound (per gm. of protein)
Aspartic[10]	133	3.4	26×10^{-5}
Glutamic[10]	147	5.8	39×10^{-5}
			Total 65×10^{-5}
Less amide nitrogen[10]	17	0.4	23×10^{-5}

Mols NaOH which should be bound, 42×10^{-5}.
Mols NaOH which one gm. of gelatin binds at pH 11, 60×10^{-5}.

Base-combining Power of Gliadin.

Amino Acids	Mol. Wt.	Per cent	Mols of NaOH which should be bound (per gm. of protein)
Glutamic[12]	147	43.7	297×10^{-5}
Aspartic[12]	133	0.2	
Tyrosine[13]	181	5.1	28×10^{-5}
β-hydroxy-glutamic[14]	163	2.4	15×10^{-5}
			Total 340×10^{-5}
Less amide nitrogen[15]	17	5.2	306×10^{-5}

Mols NaOH which should be bound, 34×10^{-5}.
Mols NaOH which one gm. gliadin binds at pH 11, 30×10^{-5}.

[11] Dunn, M. S., and Lewis, H. B., *J. Biol. Chem.*, 1921, xlix, 327.

[12] Osborne, T. B., and Guest, H. H., *J. Biol. Chem.*, 1911, ix, 425.

[13] Cross, R. J., and Swain, R. E., *J. Ind. Eng. Chem.*, 1924, xvi, 49.

[14] Dakin, H. D., *Biochem. J.*, 1919, xiii, 398.

[15] Osborne, T. B., Van Slyke, D. D., Leavenworth, C. S., and Vinograd, M., *J. Biol. Chem.*, 1915, xxii, 259.

show absolute agreement between the amount of alkali which was experimentally found to be taken up by the proteins and that which was calculated on the basis of the free dibasic acid groups, the values nevertheless are of the same magnitude and indicate at least from the qualitative standpoint that there is a distinct relationship between the alkali-combining power of these proteins and their content of free dibasic acid groups. The greatest discrepancy is found in gelatin. This, however, is not serious especially since it is very probable that in the estimation of small amounts of aspartic and of glutamic acid considerable experimental error enters.

Our method of estimating the base-combining power of the proteins was carried out according to the procedure which has previously been used by Tague[16] for amino acids and by Loeb and Hitchcock for proteins. On account of the logarithmic increase in pH on addition of alkali the method is not capable of a very high degree of accuracy at high alkalinity.

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Reactions of the urinary bladder in canine anaphylaxis.

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As an index to possible smooth muscle reactions, we have studied the pressure changes in the urinary bladder during canine anaphylactic shock, with parallel tracings of the changes in the arterial blood pressure. The dogs were sensitized to horse serum by Weil's method.[1] They were tested by intravenous injections of 0.5 to 2.0 cc. horse serum per kg. of body weight, about twenty-one days after the final sensitizing dose. The intracystic pressure was recorded by means of a glass catheter (perineal incision, male dogs). The abdomen was opened to avoid errors

[16] Tague, E. L., *J. Am. Chem. Soc.*, 1920, xlii, 173.

[1] Weil, R., *J. Immunol.*, 1923, viii, 233.