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ON THE SWELLING OF PROTEIN COLLOIDS.

A REPLY TO PROFESSOR MARTIN H. FISCHER.

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Recently, in THIS JOURNAL, Professor Martin Fischer and his collaborators have returned to the problem of the swelling of colloids under the influence of solutions of varying acidity and alkalinity.[1] The results of these new investigations, though interpreted by Professor Fischer as favorable to his views upon the physico-chemical conditions of the distribution of water in colloidal systems, and upon the nature of edema, nephritis and uremia, and other pathological states, have hardly received from their authors the necessary quantitative mathematical analysis.

A casual inspection of the data of these papers shows that they prove, or rather confirm, the established fact that the necessary and sufficient condition for great swelling in such systems as Professor Fischer has studied is a high concentration of hydrogen or hydroxyl ions.

Further discussion of the results is rendered difficult by the fact that the authors have omitted to state the hydrogen-ion concentrations of their solutions. The abscissas of their geometrical diagram are merely the serial numbers assigned to their several experiments, while reference to the data shows that it is often impossible to estimate the concentration of ionized hydrogen in the solutions. Luckily, however, it is easy to calculate with sufficient accuracy this concentration in all cases where the values approach those that have been obtained for blood or for the other fluids of the organism (except gastric juice).

In Table I are assembled the data of Fischer and Hooker upon the swelling of gelatin plates in solutions of electrolytes where the hydrogen-ion concentrations even remotely approximate such concentrations as are known to occur within the organism, even in the most extreme pathological conditions. Whenever an accurate estimate is possible the values of these concentrations, expressed as p_H, are recorded.

Before considering the significance of the data below, it should be noted that the swelling of gelatin plates in pure water, as recorded among the data of these same experiments, indicates that a large variation in the ex-

acid to determine the olefins. His plan of analysis, however, is directed only to the final determination of the % of aromatic hydrocarbons present, which is really determined by the final fractional distillation of the refined and steam rectified oil. Egloff does not in any way indicate that the high boiling residues contained in the refined oil are in large part derived from polymerization of lower boiling olefins. *Met. Chem. Eng.*, 17, 262 (1917).

[1] Fischer and Hooker, THIS JOURNAL, 40, 272 (1918); Fischer and Benzinger, *Ibid.*, p. 292; Fischer and Coffman, *Ibid.*, p. 303.

TABLE I.—DATA OF FISCHER AND HOOKER.

A. Phosphate Mixtures.

Ratio. $\frac{NaH_2PO_4}{Na_2HPO_4}$ →		10:0.	8:2	6:4	4:6	2:8	0:10
Approximate pH →		?	6.3	6.7	7.0	7.4	?
Experiment IV.....	24 hours	7.42	8.21	8.44	8.50	8.90	8.61
	48 hours	8.28	9.31	9.49	9.56	10.05	9.85
Experiment V.....	18 hours	5.15	7.65	8.00	8.42	8.55	8.48
	42 hours	6.88	9.14	9.80	10.24	10.50	10.31
Experiment VI.....	22 hours	6.28	7.08	7.50	7.90	8.16	8.68
	45 hours	7.10	8.03	9.14	9.09	9.30	10.06
Experiment VII.....	20 hours	5.90	7.11	7.65	7.50	8.02	8.17
	44 hours	6.78	8.44	9.00	9.00	9.45	9.70
Experiment VIII....	20 hours	7.10	8.68	9.35	9.49	9.81	10.23
	44 hours	8.27	10.27	11.16	11.31	11.57	12.14
Mean, 33 hours		6.92	8.38	8.95	9.10	9.43	9.62

B. Citrate Mixtures.

Ratio. $\frac{\text{Disodium citrate}}{\text{Trisodium citrate}}$ →		10:0	8:2	6:4	4:6	2:8	0:10
Approximate pH →		?	5.1	5.3	5.6	6.0	?
Experiment XIII....	24 hours	8.02	8.05	8.22	8.26	8.19	8.16
	48 hours	8.93	8.96	8.80	9.23	9.34	9.03
	72 hours	9.46	9.50	9.48	10.02	9.54	9.41
Mean, 48 hours		8.80	8.84	8.83	9.17	9.02	8.87

The data represent gain in parts of one part gelatin.

tent of the swelling of parallel experiments is to be expected. This may be illustrated by the data for the swelling in 24 hours, that period of time which yields the most satisfactory agreement.

TABLE II.

Experiment.	I	II.	III.	IV	X.	XI.	XII.	XIII.
Swelling.....	7.60	7.41	6.52	6.86	5.94	5.92	5.98	6.35

A comparison of these two tables shows that near the neutral point there is at most a very small change in the swelling of gelatin plates accompanying changes in the concentration of acid and base in the solution, or in union with the protein. Such changes as seem to be indicated by the data in question involve a slight diminution in swelling with increasing acidity or diminishing alkalinity. This conclusion also merely confirms the results of previous experiments.[1]

The experiments of Fischer and Benzinger on the swelling of fibrin also agree with previous observations upon the same subject.

Professor Fischer is therefore confronted with the situation that his latest experiments, when clearly stated, fully confirm the facts that have been employed by others to controvert his theoretical conclusions. If we may quote his own words, "Irrespective of the manner in which these

[1] Cf. Henderson, Palmer and Newburgh, *J. Pharmacol.*, **5**, 449 (1914).

mixtures are prepared (whether by progressive substitution of one salt for another, through the addition of the requisite acid to an alkali, through the addition of alkali to the proper acid, or through the addition of either acid or alkali to a given salt) it is found, when amount of water is plotted on the vertical and change in composition of the mixture on the horizontal" that for such conditions as are known to occur in the body the curve is approximately a horizontal straight line.

It is from these experiments that Professor Fischer now draws the following conclusions: "These findings are held to be applicable to the problem of water absorption by protoplasm and to sustain the old contention that even in the presence of buffer salts, there is an increase in water absorption (increased turgor or edema) with every increase in the acid (or alkali) content of the protein colloids found in the involved cell, organ or organism."

But the findings do in fact show that in the presence of the buffer mixtures of protoplasms, which alone are relevant to the question, there is little or no change in water absorption with change in the acid (or alkali)

TABLE III.—CONCENTRATION CELL MEASUREMENTS ON SOLUTIONS OF HYDROCHLORIC ACID CONTAINING GLUTENIN.

Experiment number.	Concentration of HCl.	Concentration of NaCl.	Concentration of glutenin.	$\frac{C_{HCl}}{C_{glutenin}}$.	pH.
520	0.0050 *N*	0	0.5%	10	2.56
531	0.0100	0	1.0	10	2.54
533	0.0100	0	1.0	10	2.60
533	0.0100	0	1.0	10	2.40
520	0.0050	0	1.0	5	3.05
527	0.0050	0.009 *N*	1.0	5	3.05
533	0.0050	0	1.0	5	3.05
533	0.0050	0	1.0	5	2.88
527	0.0033	0.009	1.0	3.3	3.39
531	0.0033	0.009	1.0	3.3	3.50
513	0.0030	0.001	1.0	3.0	3.37
533	0.0030	0	1.0	3.0	3.50
533	0.0030	0	1.0	3.0	3.40
520	0.0100	0	4.0	2.5	3.83
520	0.0050	0	2.0	2.5	3.78
533	0.0020	0	1.0	2.0	3.85
533	0.0020	0	1.0	2.0	3.77
513	0.0020	0.0015	1.0	2.0	3.82
514	0.003	0.001	2.0	1.50	3.99
520	0.0050	0	4.0	1.25	4.48
533	0.0010	0	1.0	1.00	4.43
533	0.0010	0	1.0	1.00	4.86
533	0.0010	0	1.0	1.00	4.67
520	0.0025	0	4.0	0.625	5.17
514	0.0010	0.002	2.0	0.5	5.14

content of the protein colloids. Not until Professor Fischer has found a colloid unlike gelatin in this respect can he hope to maintain this part of his argument.

The question of the entrance of acid into the gelatine plates involves another problem. But these experiments also support the view that the acid or alkali content of the protein colloids is a function of the hydrogen ion concentration of the solution, a theory which has perhaps never been doubted by any one but Professor Fischer. It is of course true that the method of preparation of the protein, the concentration and nature of the electrolytes in the solution, and a variety of other factors, must somewhat influence the heterogeneous equilibrium, and might conceivably influence it greatly. This, however, is a question which can only be investigated experimentally. Fischer's data point to no such large effect. The preceding unpublished data of our own upon the equilibrium between a very pure preparation of glutenin and hydrochloric acid also strongly indicate that the hydrogen-ion concentration of the solution is the important factor in determining the amount of acid taken up by the protein.

These results are reported graphically upon the accompanying diagram. The fact that the ratio of acid to protein plotted against the values of p_H yields a smooth curve may be regarded as an indication that the protein behaves like the solution of a polyvalent base, acid, or amphoteric substance.

From Fischer's own data, from those above reported, which bear more directly upon the problem, and from the known facts concerning the isohydric points of proteins, it seems certain that, as a rule, the amount of acid or base combined with most simple proteins within the physiological or pathological ranges of reaction are low and liable to little variation. Not until Fischer has found in the tissues sufficient quantities of proteins of a quite different avidity for bases or acids, can he maintain this part of his argument.

It is in vain that Professor Fischer seeks to establish a simple account of such diseases as nephritis, or such conditions as edema and uremia. No doubt simple conditions are involved in these complex conditions, but there is at present no way of conceiving them simply, except by shutting the eyes to the facts. Fischer has completely failed to establish his thesis, because it is well known that acidosis is a variable phenomenon in these conditions, and also in many others which are not contemplated by his theory.

Finally, it is simply not permissable to disregard the accurate quantitative measurements which have been accumulated in many laboratories of biological chemistry, of physiology, and of clinical medicine during the last decade. On the basis of Fischer's speculations the theory of the regulation of breathing, one of the most splendid achievements of modern physiology, which has stood the test of aviation, of gas warfare, and of

military medicine, is almost meaningless. Yet it is a fact that the composition of the blood determines the activity of the respiratory centre so as to adjust the hydrogen-ion concentration of the blood at a constant point.[1] This is only one of many facts which Professor Fischer has always disregarded.

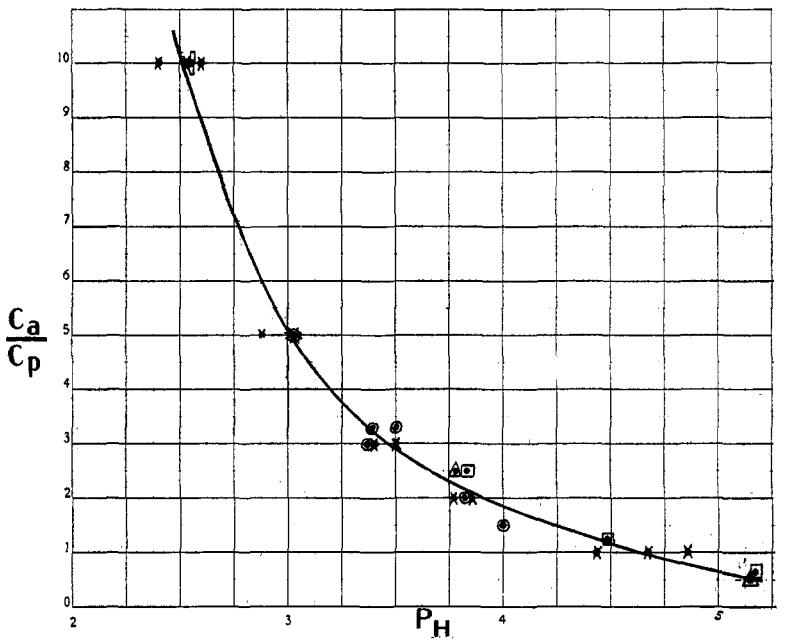

Fig. 1.—Concentration cell measurement on solutions of hydrochloric acid containing glutenin.

$\frac{C_a}{C_p}$ = Ratio of normal concentration of acid to per cent. concentration of protein.

$P_H = \log C_H$.

0.5% glutenin; 1.0% glutenin; 1.0% glutenin containing salt; 2.0% glutenin; 4.0% glutenin.

Questions of this kind, however, are not merely of scientific interest, for medical practice is also involved. Therefore in view of the above discussion, we feel bound once more to protest against therapeutic measures founded upon theories which are often inconsistent with, and always unsupported by, the established facts of chemistry and physiology.

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[1] Cf. Haldane, "Organism and Environment as Illustrated by the Physiology of Breathing," New Haven, 1917; and Barcroft, "The Respiratory Function of the Blood," Cambridge, England, 1914.