[CONTRIBUTION FROM THE EICHBERG LABORATORY OF PHYSIOLOGY IN THE UNIVERSITY OF CINCINNATI.]

THE COLLOIDAL-CHEMICAL THEORY OF WATER ABSORPTION BY PROTOPLASM.

A FIFTH RESPONSE TO SOME CRITICISMS.

BY MARTIN H. FISCHER.

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The Editor and Professor Lawrence J. Henderson have kindly permitted me to see the preceding article in manuscript form and to make reply.[1]

To avoid misunderstanding I shall in the following quote directly those pieces of Henderson's latest article which possess sufficient body to make response possible.

Henderson opens as follows:

"Recently .... Fischer and his collaborators have returned to the problem of the swelling of colloids under the influence of solutions of varying acidity and alkalinity."

I have in all my writings scrupulously avoided the use of just such terms as "acidity" or "alkalinity," employing such non-committal terms as "acid or alkali content" just because the terms "acidity" and "alkalinity" are variously defined by different authors (in terms of titration value by the old school, or in terms of hydrogen- or hydroxyl-ion concentration by the modern physical chemists) and because, as I have so many times insisted upon before, the effects of acids and of alkalies nowhere parallel either of these acidities or alkalinities either in the physiological behavior of tissues (like their water absorption) or in such simple processes as the absorption of water by protein colloids.

Henderson continues:

"A casual inspection of the data of these papers shows that they rather confirm the established fact that the necessary and sufficient condition for great swelling in such systems is the high concentration of hydrogen or hydroxyl ions."

It all depends upon what one means by "great" swelling or "high" concentration. In the papers under criticism, as well as in my older studies, it is shown that simple proteins in the presence of acids or alkalies may easily absorb a hundred times (10,000%) their dry weight of water. It must be clearly understood, however, that no such degrees of swelling are ever necessary to account either for the normal amounts of water held by cells or tissues or the excessive amounts which they may contain in even extreme grades of edema. The greatest edema that I have ever been able to produce *experimentally* in an *isolated* organ amounted to less than

[1] See also the previous criticisms of L. J. Henderson and his co-workers, *J. Biol. Chem.*, **13**, 404 (1913); *Arch. Int. Med.*, **12**, 146, 163 (1913); *J. Pharmacol.*, **5**, 449, 466 (1914); answer may be found to these in my "Edema and Nephritis," 2nd Ed., 633, 650, New York, 1915.

250% of the moist weight of the tissue. No such edemas can, however, be produced in whole animals and have the animals live. A maximum obtainable in frogs was 60% and extreme grades of edema in human beings rarely run above 25% of the normal weight of the individual. Such edemas moreover can be tolerated only in the extremities or in organs where swelling goes on unhampered by capsules or bony outer structures. An edema of the brain amounting to an increase in weight of but 5% leads to stupor, and an edema of this organ amounting to 8% results in death.

I introduce these figures because Henderson's criticisms would lead one to believe that something approximating the first figures would have to be obtained in order to explain the swelling characteristic of various "diseases" (nephritis, glaucoma, "uremia," edema of the brain, etc.) instead of the lowest figures.

How easily the biologically necessary per cents. may be covered by a slight shifting in the acid or alkali content of the tissues may be seen from Henderson's own revision of my experimental findings as put together in Table I of his paper. I pass over certain obvious objections which may be launched against this method of his, such as his ignoring of concentration of the phosphate mixtures, his working in averages, etc. In spite of this, his rearrangement of my tables does not obscure the fact that with changes in the acid or alkali content of the mixtures there is an increase in swelling amounting from a few per cent. to 50% of the moist weight of the gelatin—enough, therefore, to cover even the extremes of water absorption observed in clinical cases of edema, and this within the realm of those changing "hydrogen-ion acidities" which Henderson himself acknowledges as "physiological."

Henderson continues:

"It should be noted that the swelling of gelatin plates in pure water ....indicates that a large variation in the extent of the swelling of parallel experiments is to be expected."

This is an attempt to discredit the significance of the increased swelling observed in Henderson's admittedly "physiological" or "neutral" zone. But, as every colloid chemist knows, these differences are largely temperature effects and are taken into account in every series of experiments by employing in each of such series one or more water controls as blanks.

Henderson writes:

"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."

As noted above this "very small change" may amount to 50%.

"Professor Fischer is, therefore, confronted with the situation that his latest experiments..........fully confirm the facts........employed by others, to controvert his theoretical conclusions."

And with the same amount of compulsion.

Henderson next inserts some perfectly correct quotations from my papers which need no comment. He continues:

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

Only several to 50%.

"The question of the entrance of acid into the gelatin 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 anyone but Professor Fischer."

I have never denied that in the entrance of acid into gelatin, in the question of the acid content of a protein and, I might add, in the problem of water absorption by protein or protoplasm the concentration of the hydrogen ions is *a* function of the process. I have always maintained, and do now, that it is not the *only* function.

Henderson next writes:

"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."

Very true, as my studies show. The "heterogeneous equilibrium" may be so violently influenced that the function of the hydrogen ions is obscured entirely. The order in which hydrochloric, lactic and sulfuric acids influence protein swelling or water absorption by tissues decreases in the sequence in which they are named, in spite of the fact that the hydrogen-ion concentration is high in the dilute aqueous solutions of the first and last acids and low in the lactic. In fact, lactic acid is almost as powerful in this regard as hydrochloric, while fibrin and other proteins swell little more in sulfuric acid than in distilled water. To these items may be added that, no matter what the concentration of any acid, addition of any neutral salt decreases the amount of the protein swelling, and this out of all proportion to any fancied decrease in hydrogen-ion acidity brought about through the added salt.

Henderson adds:

"This, however, is a question which can only be investigated experimentally. Fischer's data point to no such large effect."

See the preceding paragraph, or any of the score of papers or books that I have published on this subject.[1]

[1] Martin H. Fischer, "Physiology of Alimentation," New York, 1907; "On the Swelling of Fibrin (with Gertrude Moore), *Am. J. Physiol.*, **20**, 330 (1907); "The Nature and Cause of Oedema," *J. Am. Med. Assoc.*, **52**, 830 (1908); "Ueber die Analogie zwischen der Wasserabsorption durch Fibrin und durch Muskel," Pflüger's *Arch. für Physiol.*,

Henderson continues:

"The following 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."

No doubt this is true but the point under discussion is not the acid taken up by a protein, but the *water*.

"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."

124, 69 (1908); "Weitere Versuche über die Quellung des Fibrins," *Ibid.*, **125**, 99 (1908); "Ueber Augenquellung und das Wesen des Glaucoms," *Ibid.*, **125**, 396 (1908); "Ueber Augenquellung und das Wesen des Glaucoms," *Ibid.*, **127**, 1 (1909); "Ueber Hornhauttrübungen," *Ibid.*, **127**, 46 (1909); "Bemerkungen zu einer kolloidchemischen Theorie des Lackfarbwerdens der roten Blutkorperchen," *Kolloid-Z.*, **5**, 146 (1909); "Ueber die Antagonistische wirkung der Neutralsalze auf die Quellung des Fibrins in Säuren und Alkalien" (with Gertrude Moore), *Ibid.*, **5**, 197 (1909); "Ueber die Entstehung und Natur des Stauungsoedem der Niere und der Leber" (with Gertrude Moore), *Ibid.*, **5**, 286 (1909); "The Relief of Glaucoma through Subconjunctival Injections of Sodium Citrate" (with Hayward G. Thomas), *Ann. Ophthalm.*, **19**, 40 (1910); "Das Oedem als kolloidchemischen Problem (nebst Bemerkungen über die allgemeine Natur der Wasserbindung in Organismen)," *Kolloidchem. Beihefte*, **1**, 93 (1910); "Ueber das Wesen der Trüben Schwellung," *Kolloid-Z.*, **8**, 159 (1911); "Weiteres zur Kolloidchemischen Analyse der Nephritis," *Ibid.*, **8**, 201 (1911); "On the Nature, Cause and Relief of Glaucoma," *Trans. Am. Acad. Ophthalm. Oto-laryng.*, 193 (1911); "Beiträge zur Kolloidchemischen Analyze der Absorptions und Secretionsvorgänge" (Die Absorption aus der Bauchhöhle), *Kolloidchem. Beihefte*, **2**, 304 (1911); "Zur Theorie und Praxis der Transfusion" (with James J. Hogan), *Ibid.*, **3**, 385 (1912); "Ueber die Konktraktilität von Katgut und die Theorie der Muskelkontraktilität" (with W. H. Strietmann), *Kolloid-Z.*, 10, 65 (1912); "Ueber die Aufnahme von Wasser durch das Nervengewebe" (with M. O. Hooker), *Ibid.*, 10, 283 (1912); "A Response to Some Criticisms of the Colloid-Chemical Theory of Water Absorption by Protoplasm," *Biochem. Bull.*, **1**, 444 (1912); "Further Remarks on the Treatment of Nephritis," *Trans. Assoc. Am. Phys.*, **27**, 595 (1912); "A Further Response to Some Criticisms of the Colloid-Chemical Theory of Water Absorption by Protoplasm," *J. Am. Med. Assoc.*, **59**, 1429 (1912); "The Colloid-Chemical Theory of Water Absorption by Protoplasm" (a third response to some criticisms), *Ibid.*, 60, 348 (1913); "The Treatment of Nephritis and Allied Conditions," *Ibid.*, **60**, 1682 (1913); "Weitere Beiträge zur Behandlung der Nephritis und verwandter Erscheinungen," *Kolloidchem. Beihefte*, **4**, 343 (1913); "Some Physicochemical Principles in Therapy," *Forchheimer's Therapeusis of Internal Diseases*, **1**, 1, New York (1913); "On the Colloid-Chemical Action of the Diuretic Salts" (with Anne Sykes), *Science*, **37**, 845 (1913); "Non-Electrolytes and the Colloid-Chemical Theory of Water Absorption" (with Anne Sykes), *Science*, **38**, 486 (1913); "Ueber die kolloid-chemische Wirkung der diuretischen Salze" (with Anne Sykes), *Kolloid-Z.*, **13**, 112 (1913); "Ueber den Einfluss einiger Nichtelektrolyte auf die Quellung von Protein" (with Anne Sykes), *Ibid.*, **14**, 215 (1914); "Ueber die Kolloidchemie der Zuckerdiurese" (with Anne Sykes), *Ibid.*, **14**, 223 (1914); etc., etc. For a running account, see my "Edema and Nephritis," 2nd Ed., New York, 1915.

Also true, but what connection does Henderson expect to have made of this, with the problem of water absorption?

"........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."

But even these "low" amounts are sufficient to account for very considerable edemas. The increased carbonic acid content of venous blood over arterial raises the "hydrogen-ion acidity" but little, yet even this is sufficient to increase the water absorption by the cells in the blood stream 5 to 15%, and if there is any interference with the free circulation of the blood, the figure jumps to 30%.[1]

"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."

Certainly not! Not even when done in my laboratory.

"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."

Why so? I happen to have admired and taught the principles established by Haldane and his followers for years past.

"Yet it is a fact that the composition of the blood determines the activity of the respiratory center so as to adjust the hydrogen-ion concentration of the blood at a constant point."

This is only partially true and then only for normal individuals as Haldane and the English clinicians have themselves shown. In cases of heart disease, respiratory disease, diabetes and other clinical conditions associated with edema the observations of these very authors show that evidences of an abnormally high acid content of the blood and tissues (as evidenced, among other things, by an abnormally high hydrogen-ion acidity of the blood) are regularly present.

"........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."

This is Henderson's opinion. Among those of another persuasion in this matter of therapy are Arthur D. Dunn,[2] Albert J. Bell,[3] Paul G. Woolley,[4] Edgar G. Ballenger and Omar F. Elder,[5] Rufus Southworth,[6] Gordon F.

[1] See H. J. Hamburger, *Osmotischer Druck and Ionenlehre*, 1, 291, 404, Wiesbaden, 1902.

[2] Arthur D. Dunn, *Lancet-Clinic*, **108**, 8 (1912).

[3] Albert J. Bell, *Am. J. Med. Sci.*, **144**, 669 (1912); personal communication (1914).

[4] Paul G. Woolley, *J. Am. Med. Assoc.*, **63**, 596 (1914).

[5] Edgar G. Ballenger and Omar F. Elder, *J. Am. Med. Assoc.*, **62**, 197 (1914).

[6] Rufus Southworth, *Lancet-Clinic*, Sept. 5 (1914).

McKim,[1] H. Lowenburg,[2] Geo. T. Grinnan,[3] H. B. Weiss,[4] J. Mitchell Clarke,[5] Herbert Brown,[6] W. deB. MacNider,[7] A. W. Sellards,[8] V. Pleth[9] and many more.

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ON THE SWELLING OF PROTEIN COLLOIDS. A REPLY.

By L. J. Henderson.

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Professor Fischer's rejoinder seems to me to call for no modification of my original statement. But, in that he has at length undertaken to consider the question of the quantitative relation between change of volume of a colloid and change of hydrogen-ion concentration, I wish to make the following additional remarks:

First, Fischer is mistaken in supposing that I have admitted or that there is in fact any evidence that the hydrogen-ion concentration in any part of the body can ever vary as widely as in those solutions of his to which attention has been called. The present state of knowledge upon variations of the hydrogen-ion concentration in the body may be illustrated by Michaelis's extensive studies of venous blood.[10] The data fall into three groups: (I) 64 measurements on 28 normal specimens; (II) 34 measurements on 17 pathological specimens from cases of diabetes, nephritis, and edema, which therefore involve conditions contemplated by Fischer's theories; (III) 38 measurements on 19 pathological specimens representing various other diseases. The data are summarized in the following table:

Table I.—Values of p_H for Venous Blood (Michaelis).

	I.	II.	III.
High	7.67	7.67	7.74
Low	7.49	7.50	7.42
Mean	7.58	7.58	7.62
Range	0.18	0.17	0.32

Evidently the values of p_H for venous blood, both in normal and in pathological conditions, are liable to very small variations. In a single case of diabetic coma just before death Michaelis observed a value of p_H of 7.12.

[1] Gordon F. McKim, personal communication (1914).

[2] H. Lowenburg, *J. Am. Med. Assoc.*, **63**, 1906 (1914).

[3] Geo. T. Grinnan, *Virg. Med. Semi-Month.*, **20**, 523 (1916).

[4] H. B. Weiss, *J. Am. Med. Assoc.*, **68**, 1618 (1917); *Ohio State Med. J.*, **13**, 595 (1917).

[5] J. Mitchell Clarke, *Brit. Med. J.*, **2**, 239 (1917).

[6] Herbert Brown, personal communication from Flanders received Sept. 1, 1917.

[7] W. deB. MacNider, *J. Exp. Med.*, **23**, 171 (1916); *Ibid.*, **26**, 19 (1917); *Proc. Soc. Exp. Biol. Med.*, **14**, 140 (1917).

[8] A. W. Sellards, "Acidosis and Clinical Methods," Cambridge, 1917.

[9] V. Pleth, personal communication, 1917.

[10] L. Michaelis, "Die Wasserstoffionenkonzentration," Berlin, **1914**, pp. 101–105.