product and purified. Both of these compounds are yellow and dissolve in alkali with an orange-yellow color.

6. Tetrabromophenoltetraiodophthalein results from the bromination of phenoltetraiodophthalein. It is a yellow crystalline product, soluble in alkali with a deep blue color.

7. The corresponding diacetate, dibenzoate, and dimethyl ether were made.

8. Tetraiodophenoltetraiodophthalein is a yellow crystalline substance soluble in alkaline solution with a deep blue.

9. Its diacetate, dibenzoate, and dimethyl ether are all yellow.

10. The absorption spectra and theoretical discussion of these substances will be reported upon in a later paper.

PITTSBURGH, PA.

THE MOLECULAR MECHANISM OF COLLOIDAL BEHAVIOR.

I. THE SWELLING OF FIBRIN IN ACIDS.

BY RICHARD C. TOLMAN AND ALLEN E. STEARN.

Received October 22, 1917.

In previous articles,[1] one of the authors has discussed the general behavior of colloidal systems from a *thermodynamic* point of view. In a series of articles, of which this is to be the first, it is proposed to subject a number of typical colloidal systems to an intensive study from a *molecular* or *microscopic* point of view. Such studies will provide a much more intimate and complete basis for the explanation and prediction of colloidal behavior than can be done on the basis of thermodynamic considerations alone. No special attempt will be made to preserve any logical order of publication for these articles.

The swelling of protein colloids in acid and in alkaline solution and the decrease in swelling produced by the addition of neutral salts is a matter, both of great physiological and pathological interest, and has been particularly studied by Fischer[2] in his important experimental and clinical researches. The purpose of the present work is to investigate the molecular mechanism by which such swellings and reductions in swelling are brought about. We may say in advance, that our experimental results are in accord with the theory that a colloidal gel, such as a piece of fibrin covered by water, is a fibrous sponge-like structure with many minute pores or pockets which are themselves full of water. The addition of acid or alkali is followed by the adsorption, respectively, of hydrogen or hydroxide ion on the surface of these pockets and a consequent increase in their size owing to electrostatic repulsion, the increase in size being accompanied, of course, by imbibition of solution. The further addition

[1] Tolman, THIS JOURNAL, **35**, 307, 317 (1913); *Science*, **44**, 565 (1916).

[2] Fischer, "Edema and Nephritis," New York, 1915.

of a neutral salt to the solution is followed by a decrease in swelling since the ions of the added salt will arrange themselves in such a way as to neutralize the original electrostatic repulsion.

Experimental Methods.

In the work which is now to be described, our method of attack has been to determine the swelling of a typical and easily handled protein colloid, blood fibrin, in solutions of hydrochloric, nitric, sulfuric, acetic, and formic acids of varying concentrations and to determine at the same time the amount of acid adsorbed from the solution by the fibrin. We have, furthermore, made similar measurements with solutions of sodium chloride and mixed solutions of acid and sodium chloride.

The Materials.—The same sample of blood fibrin was used throughout the work. It was purified before use by grinding to a coarse powder and washing repeatedly with 0.1 *N* hydrochloric acid. It was then washed with distilled water until the acid concentration in the wash water had fallen to less than 0.0001 *N*, and the water drained off. It was then dried in a current of warmed air for several days to volatilize any remaining acid.

The acids and salt used were ordinary "C. P." reagents.

The Swellings.—In order to determine the swellings of the fibrin, a number of special test tubes were procured of a *uniform cross section* of one-half inch and with *flat bottoms*. Uniform samples of 0.3 g. each of the powdered fibrin were put in these tubes and 15 cc. of the desired solution added and the heights to which the fibrin rose compared with a water control. In general, the samples were allowed to stand twenty-four hours with occasional shaking, this being long enough for the final height to be attained. (On further standing, a slight settling usually occurred.)

The Adsorption.—The adsorption experiments were carried out in glass "finger bowls," usually 2 g. of fibrin being used and 100 cc. of solution added, the materials thus being in the same proportions as in the swelling experiments. The solutions were allowed to stand twenty-four hours with occasional stirring and then analyzed. The acids were titrated against sodium hydroxide, standardized with oxalic acid, and the sodium chloride determined by Volhard's method, using ferric nitrate as an indicator. While the adsorption was taking place the finger bowls were kept covered with closely fitting cover glasses, which did not permit enough evaporation seriously to affect the results. In further work glass-stoppered bottles will be used.

The number of mols of acid A *adsorbed per g. of fibrin* was calculated by the following formula:

$$A = \frac{(C_o - C)V}{W} \qquad (1)$$

where C_o is the original concentration of the solution in mols per liter, C the reduced concentration after the adsorption has taken place, V the volume in liters of solution employed, and W the weight of fibrin in grams.

Experimental Results and Discussion.

The Strong Acids.—The three *upper* curves in Fig. 1 show the swelling of fibrin in different concentrations of hydrochloric, nitric and sulfuric acids. The ordinates give the height in mm. to which the fibrin rose and the abscissas give the concentrations C in mols per liter of the surrounding

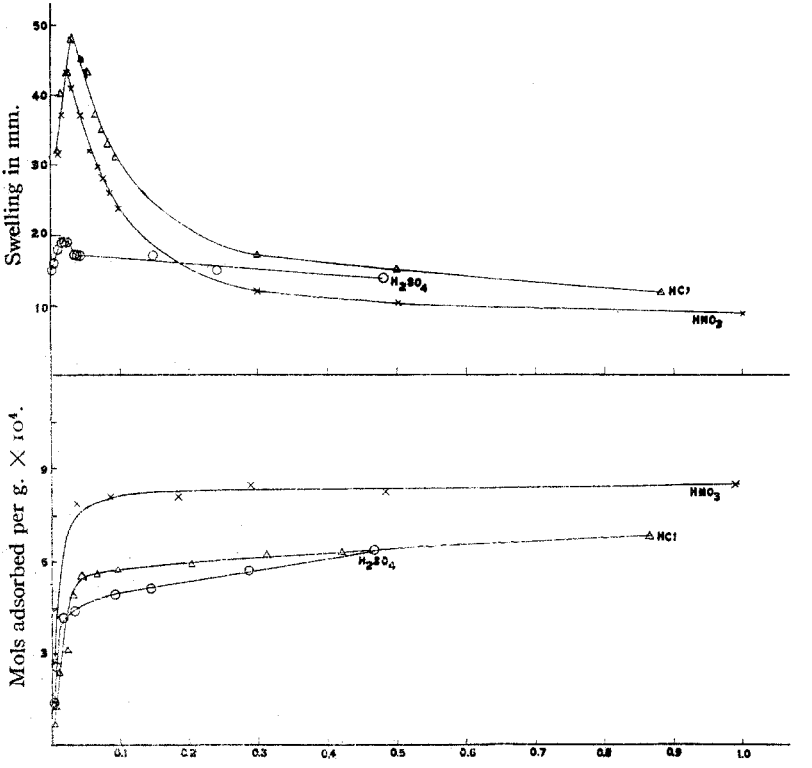


Fig. 1.

solution (after adsorption has taken place). The sharp maximum of swelling at a low concentration of acid is to be noticed. The three *lower* curves in Fig. 1 show the adsorption of acid by the fibrin. The ordinates give the number of mols of acid adsorbed per g. of fibrin and the abscissas give the concentrations of solution plotted on the same scale as above. Actual observations are indicated in these plots by points, and considering the lack of precision and reproducibility in colloidal behavior, we believe that the smooth curves give a reasonably good representation of the facts.

It will be noticed that the adsorbed acid at first increases very rapidly with the concentration and then more slowly finally approaching nearly a constant value. This accords with the usual behavior of adsorption curves, and could be explained by the assumption that the adsorption proceeds until the surface in question finally becomes coated with a layer of adsorbed substance one molecule thick and adsorption then ceases.[1]

In the particular case at hand, we believe that the walls of the pockets or pores in the interior of the fibrin have a great affinity for hydrogen ion, or in accordance with the amphoteric nature of the amino acids composing the fibrin, also for hydroxide ion. As soon as a little acid is added, hydrogen ion is at once adsorbed on the surface of these pockets, the corresponding anions being held by electrostatic attraction in the neighborhood of the adsorbed hydrogen, to form a "double layer." The adsorption of hydrogen ion continues until about 0.0006 to 0.0008 mol of hydrogen ions per g. of fibrin have been adsorbed, the surface of the pockets then being pretty well covered. Further addition of acid to the solutions then results merely in increasing the concentration of acid in the interior of the pockets and does not appreciably increase the amount adsorbed.

The course followed by the adsorption can apparently be used to explain the course taken by the swelling. On the first additions of acid when the amount of adsorbed hydrogen ion is increasing rapidly, the swelling also increases rapidly, owing to the electrostatic repulsion which forces the walls of the pockets further apart with the imbibition of solution. Later additions of acid, however, serve to increase the concentration of the acid in the interior of the pockets without appreciably raising the amount of adsorbed hydrogen ion, and this is accompanied by a decrease in swelling since, in accordance with electrostatic theory, the ions in the interior of the pocket will evidently tend to arrange themselves in such a way as to neutralize the original electrostatic repulsion. We believe that this accounts for the decided maximum of swelling in the particular range of concentration where the amount of adsorbed acid ceases to rise rapidly.

The Weak Acids.—The two upper curves in Fig. 2 show the *swelling* of fibrin in different concentrations of acetic and formic acids and the two lower curves show the *adsorption* of the acid. For purposes of easy comparison these curves are plotted on the same scale as in the previous figure for the strong acids.

Especial attention may be called to the fact that there is no *maximum* swelling in the case of the weak acids. In terms of our theory, this arises because we are working with a weak electrolyte and there is only a small concentration of ions in the interior of the pockets whose electrical fields

[1] See for example Langmuir, THIS JOURNAL, 38, 2221 (1916).

are available for neutralizing the electrostatic repulsion of the adsorbed ions.

This difference between the behavior of weak and strong acids becomes very noticeable if we go to high concentrations. Since there is no strong electrolyte present to bring about a reduction of swelling, we found at high concentrations of acetic acid (say, from 0.5 N on), that the fibrin was swollen into a sticky gelatinous mass. Moreover, we found that the amount of adsorbed acid continued to increase with increasing concen-

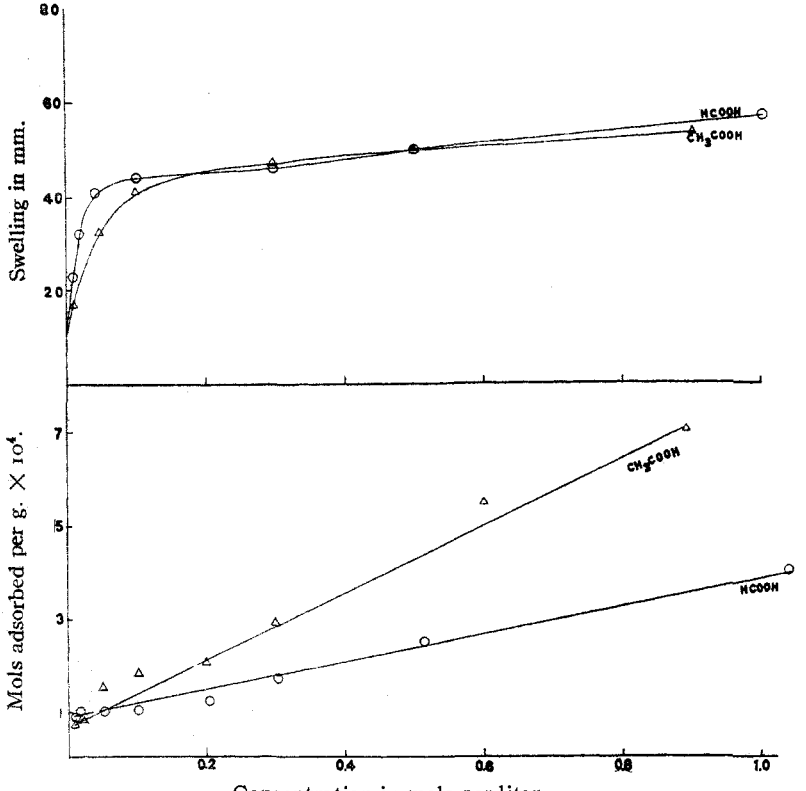


Fig. 2.

tration, until finally with the N acetic acid we got in the neighborhood of 0.006 mol of acid adsorbed per g. of fibrin as against the maximum adsorption with hydrochloric acid apparently of about 0.0007 mol per g. We are inclined to believe that this is due to the fact that the continued swelling leads to a sort of opening up of the pockets or separation of fibers which furnishes new surfaces where adsorption can take place.

Mixed Acid and Salt Solutions.—We have explained the occurrence of a maximum swelling in the case of strong acids by assuming, at the

higher concentrations, that the strong electrolyte in the interior of the pockets furnishes ions which tend to arrange themselves in such a way as to neutralize the repulsive forces exerted by the adsorbed ions. If this is the case, we should obtain a check on our theory by investigating the behavior of fibrin in acid solutions to which strong *neutral* electrolytes are added.

This behavior in mixed solutions is given in Fig. 3, where the two upper curves give the *swelling* of fibrin in solutions which were made up

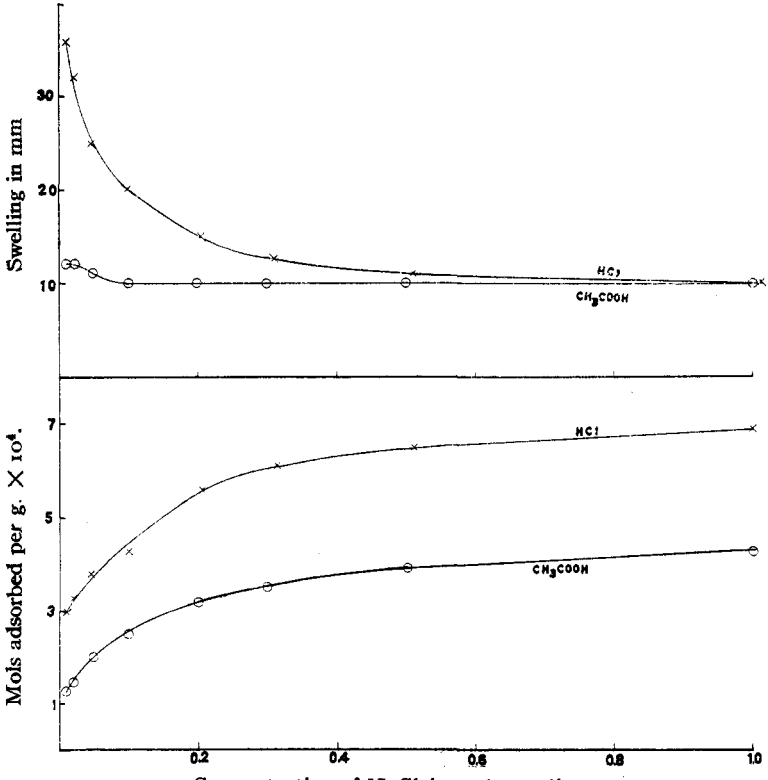


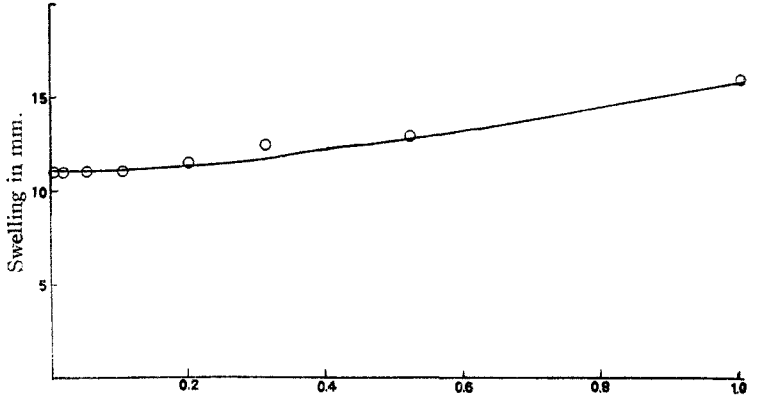
Fig. 3.

0.015 *N*, respectively, with hydrochloric and acetic acids, and with increasing concentrations of sodium chloride as indicated by the abscissas of the plot. It will be noticed in agreement with our theory that increasing the concentration of the added salt reduces the swelling produced by the acid until at the higher concentrations the swelling is even less than that in pure water in the case of both the acids.

We must also call attention to the important effect which the added salt has upon the *adsorption* of acid. This is shown by the two lower curves

in Fig. 3. It will be seen that increasing the concentration of *salt* increases the adsorption of *acid*. This also seems explainable in terms of our concepts. As soon as any hydrogen ion is adsorbed this sets up a directed electric field which, as we have already pointed out, leads to an increase in size of the pockets, but will also evidently have the effect of discouraging further adsorption. The neutralization of this field by the ions of the neutral salt would increase the adsorption, as actually turns out to be the experimental fact. (It should be noticed that sodium chloride increases the adsorption of acetic acid as well as of hydrochloric acid, thus ruling out the common ion effect as an explanation.)

Pure Salt Solutions.—We may now finally call attention to the behavior of fibrin in pure sodium chloride solutions. There is, of course, no great swelling in such solutions since our theory does not lead us to expect any selective adsorption either of sodium or chloride ion. This is shown by the curve in Fig. 4, where it will be seen that even at N concentration



Concentration of NaCl in mols per liter.

Fig. 4.

the swelling is still small, what there is, probably being due to a slight specific adsorption either of sodium or chloride ion.

Analysis of the solutions gave further evidence that there is no particular adsorption of the ions of sodium chloride, since the concentration of the solutions actually rose when they were placed in contact with fibrin, thus showing that there is more tendency for water to be adsorbed by the fibrin than for sodium chloride. In fact, analyses of this kind would permit us to calculate the amount of adsorbed water, making, of course, the somewhat improbable assumption that absolutely no salt at all is adsorbed. In some further work now in progress it is hoped to obtain more satisfactory information as to the amount of adsorbed water, using sugar as the non-adsorbed reference substance.

Conclusion.

We believe the data and discussion which we have presented are sufficient to make our theory as to the molecular mechanism of protein swelling highly probable. A brief restatement of the theory will not be out of place.

(1) In accordance with their amphoteric nature protein colloids have a marked tendency to adsorb hydrogen ion from acid solutions and hydroxide ion from alkaline solutions.

(2) In an acid solution, the adsorbed hydrogen ions, together with a corresponding number of anions, form a "double layer" on the walls of the pockets or pores in the interior of the gel and this leads to swelling and imbibition of water by electrostatic repulsion.

(3) The addition of a strong electrolyte to such a swollen colloid, either a neutral salt or excess of the strong acid which caused the original swelling, will furnish ions in the interior of the pockets which will tend to arrange themselves so as to neutralize the electrical fields of the adsorbed layer and thus bring about a reduction of swelling.

(4) The addition of a neutral salt to an acid solution tends to neutralize the electrical field of the adsorbed acid, and hence makes it easier for more acid to get to the surface of the pockets, thus leading to increased adsorption.

(5) It must be further noticed that in accordance with our theory, salts with polyvalent ions should be more effective in reducing swelling than salts with univalent ions; since, for example, a bivalent ion will take up no more room than a univalent ion, but will be twice as effective in neutralizing an existing electric field. That salts with polyvalent ions are the most effective in producing dehydration has been shown, particularly by the extensive work of Fischer.[1]

The conclusions which we have drawn are largely based on the experimental data for fibrin which we have presented in this paper. We have also made some experiments, however, on slabs of gelatin, and obtained an adsorption curve for gelatine and hydrochloric acid quite similar to the one for fibrin. We may further call attention to the similar adsorption curves obtained by Herzog and Adler,[2] using powdered hide and the work of Procter[3] using gelatin.

We have spoken throughout the article of the adsorption of hydrogen ion (or hydroxide ion). We have purposely made no assumptions, however, as to the molecular nature of this adsorption. Our theory would be equally applicable, if the adsorption process should lead to a fairly uniform coating of ions over the whole of the exposed surface, or, on the other hand, if the hydrogen ion should only tend to go on to the protein

[1] *Loc. cit.*

[2] *Kolloid-Z.*, supplement March, **1908**, p. 111.

[3] *J. Chem. Soc.*, **105**, 313 (1914).

molecule at special points, where there is a particularly strong stray field, say, for example, where the amino and acid groups of the amino acids come together. It may be that later work will throw light on this point.

URBANA, ILL.

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

ON THE SWELLING OF GELATIN IN POLYBASIC ACIDS AND THEIR SALTS.[1]

BY MARTIN H. FISCHER AND MARIAN O. HOOKER.

Received October 31, 1917.

I. Introductory.

It is now safe to conclude that the amount of water absorbed by any living cell, organ or organism, is essentially dependent upon its content of hydrophilic colloids and the state of these. As proof for this may be cited the complete analogy which exists between the laws governing the absorption of water by protoplasm and those governing the same phenomenon as observed in various simple colloids—more particularly the proteins—under physiological and pathological conditions.[2] On such a colloid-chemical basis it is now easy to account for the fairly constant amount of water held by living cells and tissues normally and so to explain, for example, their so-called normal "turgor." But under pathological conditions both in plants and in animals this normal degree of hydration may be much increased, when we have before us the "excessive turgor" characteristic of various plant diseases, or the edema of the animal pathologists as this involves individual cells, organs, or the body as a whole. Edema may, in other words, be defined as a state of excessive hydration of the body colloids, while as "causes" of the edema may be cited substances or conditions which under the circumstances prevailing in living protoplasm are capable of increasing its water holding powers to beyond the normal limits.

In connection with this problem of the "causes" of edema a study of the conditions which under laboratory circumstances increase the hydration capacity of different colloids has again yielded valuable hints as to the nature of those which are active in living protoplasm. Among the substances thus active so far as the proteins are concerned, acids occupy a first place; important, too, are the amines; while effective in this regard,

[1] A preliminary statement of the results detailed in this paper appeared in *Science*, 46, 189 (1917).

[2] Martin H. Fischer, "Physiology of Alimentation," 250, 268, New York, 1917; *Am. J. Physiol.*, 20, 339 (1907); *Pflüger's Arch.*, 124, 69 (1908); for a running account and detailed references, see Fischer, "Edema and Nephritis," 2nd Ed., New York, 1915.