

amount of water thus held and this independently of the fact that such accumulation of acid may occur in the presence or in the absence of so-called "buffer" salts. Through the accumulation or production in protoplasm of an abnormally great amount of acid (or of alkali), we are thus enabled to explain the mechanism by which the abnormally high hydrations of living cells are brought about as such are observed in the excessive turgors of plant tissues, in the edemas which involve the animal body, or in those "diseases" which are in essence only edemas of certain organs, like nephritis (edema of the kidney), glaucoma (edema of the eye), or "uremia" (edema of the brain).

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

ON THE LIQUEFACTION OR "SOLUTION" OF GELATIN IN POLYBASIC ACIDS AND THEIR SALTS.

By MARTIN H. FISCHER AND WARD D. COFFMAN.

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I. Introduction.

The importance of the swelling and of the liquefaction or "solution" of a protein colloid for the interpretation of numerous biological or technological processes has been repeatedly emphasized.¹ Since the laws which govern the absorption of water by simple colloids (like the proteins) and those which govern the absorption of water by animal and plant tissues are identical, it is now easy to explain upon a colloid-chemical basis both the normal and the abnormal water contents of cells and tissues as observed under physiological and pathological circumstances and variously designated turgor, plasmoptysis and edema. On the other hand, the changes characteristic of the liquefaction or "solution" of a previously solid colloid (as when gelatin liquefies with rise of temperature) may be and have been called upon to explain the "solution" of some or all of the colloid constituents found in cells, thus accounting for the "softening" of the involved organs as well as for the appearance of the traces of colloids found in many normal "secretions" or the larger amount of such colloids found "dissolved" under pathological circumstances in the fluids bathing swollen or edematous tissues as exemplified, for instance, in the higher albumin content of urine coming from a swollen kidney, in that of the cerebral fluid covering a swollen brain, etc.

More simply stated, excessive turgor, plasmoptysis and edema are therefore to be defined as states of increased hydration (solvation) of the body colloids; and albuminuria, excessive protein content of spinal cord fluid, etc., as states of increased "solubility" of these colloids.

¹ Martin H. Fischer, "Edema and Nephritis," Ed. 2, New York, 1915, where references to the older literature on this subject may be found.

The causes which may bring about a swelling of the involved tissues or their liquefaction or "solution" may in turn be found in those circumstances which are capable of bringing about these physicochemical changes in such colloids as constitute protoplasm. As of dominant importance in this matter an abnormal production or accumulation of acid in the pathologically involved tissues has been given first emphasis, though this is not by any means to be looked upon as the only cause for the observed colloid-chemical changes. In the case of the proteins, for example, the alkalis, the amines, pyridin and urea produce the same types of changes.

Because in protoplasm increased hydration (solvation) and liquefaction or "solution" of the involved colloids are almost constantly associated with each other it was suggested from the first that a common cause undoubtedly lay behind both processes. This conclusion has been repeatedly verified both in experiments upon simple colloids and upon animals. Proteins, for example, not only swell and go into solution under the influence of acids, but acid intoxications in animals not only make these swell (develop an edema) but cause their organs to "dissolve" (make albumin appear in the urine, for instance).

It is a commonly accepted view that this liquefaction or "solution" of a protein is but the extreme of what in lesser degree is called swelling, but, as pointed out in previous papers¹ *the swelling of a protein and its liquefaction or "solution" are totally different processes*. When one works with gelatin at concentrations and temperatures near its gelation or melting point, it can easily be shown that *the phenomena of hydration (swelling) and of "solution" while frequently associated are essentially different*. *Hydration is to be regarded as a change through which the protein enters into physicochemical combination with its solvent (water); "solution," as one which can be most easily understood at the present time as the expression of an increase in the degree of dispersion of the colloid*.

Since our previous experiments on the liquefaction or "solution" of gelatin in acids or alkalis with or without the simultaneous presence of various neutral salts might have voiced against them the same criticisms which have been raised against our experiments on swelling, namely, that the effects of the acids and alkalis were not tried out in the presence of "buffer" salts and so could not be applied to the living organism, we ran experiments on the solution of gelatin in parallel with those previously published² on the swelling of gelatin in various polybasic acids and their salts. As the following experimental facts show, *there is a progressive increase in the tendency of gelatin to go into solution in mixtures of the salts*

¹ Martin H. Fischer, "Edema and Nephritis," Ed. 2, 433, 444, New York, 1915; *Science*, 42, 223 (1915); *Kolloid Z.*, 17, 1 (1915).

² Martin H. Fischer and Marian O. Hooker, *Science*, 46, 189 (1917).

of polybasic acids as the amount of acid or alkali in these mixtures is increased from a given low point.

II. Experiments.

The same gelatin was used in these experiments as was used in our previous ones. Its quality was of such high grade that an 0.8% solution of the stock gelatin would set into a solid mass when left to itself for a few hours at 25°. To make sure of a stiff gelatin mixture we used a concentration well above this, *viz.*, 1% gelatin and set the thermostat for 20°. It should be added that all the tubes and their contents were treated in exactly the same fashion as to methods of mixing, exposure to heat or other influences which might change temporarily their gelation characteristics, etc.

In Table I are shown the effects of a progressive change from the extreme of a pure phosphoric acid through equimolar concentrations of mono-, di- and trisodium phosphate to pure sodium hydroxide upon the physical state of a fixed amount of gelatin contained in a unit volume of solvent. The maintenance of solidity by the gelatin in the middle of the series with progressive increase in fluidity to the left or to the right of this middle point can be more easily observed in the actual experiments than can be described in words or shown in such a photograph as that of Fig. 1. From

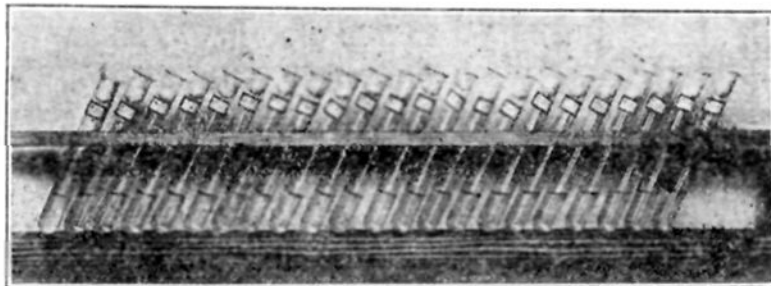


Fig. 1.

so stiff a gelatin that it vibrates when the tube is touched in the middle of the series, we pass through gelatins on either side of this which show the first evidences of a tendency to flow, to the end members which are almost as fluid as thin soup. The change from the solid to the fluid state may be observed in Fig. 1 by noting the line of the meniscus in the tubes; while on slanting the tubes this remains fixed and therefore forms an angle with the horizontal in the middle of the series, it assumes a horizontal position as we approach either end.

Since the concentration of the phosphate in Table I is about ten times that observed, for instance, in the tissues of the human body, we did a second series at a lower concentration of the phosphate and at one more nearly corresponding to physiological conditions. The results so far as maintenance of solidity or liquefaction of the gelatin is concerned are,

TABLE I.
Gelatin—Phosphoric acid through phosphates to sodium hydroxide.

Solution number.	Mixture.	Physical state.
(1)	5 cc. 2% gel. + 5.0 cc. H ₂ O (control).....	solid
(2)	5 cc. 2% gel. + 1.0 cc. 1 <i>N</i> H ₃ PO ₄ + 4.0 cc. H ₂ O.....	liquid
(3)	5 cc. 2% gel. + 0.8 cc. 1 <i>N</i> H ₃ PO ₄ + 0.2 cc. 1 <i>M</i> monosod. phos. + 4 cc. H ₂ O.....	liquid
(4)	5 cc. 2% gel. + 0.6 cc. 1 <i>N</i> H ₃ PO ₄ + 0.4 cc. 1 <i>M</i> monosod. phos. + 4 cc. H ₂ O.....	semi-solid
(5)	5 cc. 2% gel. + 0.4 cc. 1 <i>N</i> H ₃ PO ₄ + 0.6 cc. 1 <i>M</i> monosod. phos. + 4 cc. H ₂ O.....	semi-solid
(6)	5 cc. 2% gel. + 0.2 cc. 1 <i>N</i> H ₃ PO ₄ + 0.8 cc. 1 <i>M</i> monosod. phos. + 4 cc. H ₂ O.....	solid
(7)	5 cc. 2% gel. + 1.0 cc. 1 <i>M</i> NaH ₂ PO ₄ + 4.0 cc. H ₂ O.....	solid
(8)	5 cc. 2% gel. + 0.8 cc. 1 <i>M</i> NaH ₂ PO ₄ + 0.2 cc. 1 <i>M</i> Na ₂ HPO ₄ + 4 cc. H ₂ O.....	solid
(9)	5 cc. 2% gel. + 0.6 cc. 1 <i>M</i> NaH ₂ PO ₄ + 0.4 cc. 1 <i>M</i> Na ₂ HPO ₄ + 4 cc. H ₂ O.....	solid
(10)	5 cc. 2% gel. + 0.4 cc. 1 <i>M</i> NaH ₂ PO ₄ + 0.6 cc. 1 <i>M</i> Na ₂ HPO ₄ + 4 cc. H ₂ O.....	solid
(11)	5 cc. 2% gel. + 0.2 cc. 1 <i>M</i> NaH ₂ PO ₄ + 0.8 cc. 1 <i>M</i> Na ₂ HPO ₄ + 4 cc. H ₂ O.....	solid
(12)	5 cc. 2% gel. + 1.0 cc. 1 <i>M</i> Na ₂ HPO ₄ + 4.0 cc. H ₂ O.....	solid
(13)	5 cc. 2% gel. + 0.8 cc. 1 <i>M</i> Na ₂ HPO ₄ + 0.2 cc. 1 <i>M</i> Na ₃ PO ₄ + 4 cc. H ₂ O.....	solid
(14)	5 cc. 2% gel. + 0.6 cc. 1 <i>M</i> Na ₂ HPO ₄ + 0.4 cc. 1 <i>M</i> Na ₃ PO ₄ + 4 cc. H ₂ O.....	solid
(15)	5 cc. 2% gel. + 0.4 cc. 1 <i>M</i> Na ₂ HPO ₄ + 0.6 cc. 1 <i>M</i> Na ₃ PO ₄ + 4 cc. H ₂ O.....	semi-solid
(16)	5 cc. 2% gel. + 0.2 cc. 1 <i>M</i> Na ₂ HPO ₄ + 0.8 cc. 1 <i>M</i> Na ₃ PO ₄ + 4 cc. H ₂ O.....	liquid
(17)	5 cc. 2% gel. + 1.0 cc. 1 <i>M</i> Na ₃ PO ₄ + 4.0 cc. H ₂ O.....	liquid
(18)	5 cc. 2% gel. + 0.8 cc. 1 <i>M</i> Na ₃ PO ₄ + 0.2 cc. 1 <i>N</i> NaOH + 4 cc. H ₂ O.....	liquid
(19)	5 cc. 2% gel. + 0.6 cc. 1 <i>M</i> Na ₃ PO ₄ + 0.4 cc. 1 <i>N</i> NaOH + 4 cc. H ₂ O.....	liquid
(20)	5 cc. 2% gel. + 0.4 cc. 1 <i>M</i> Na ₃ PO ₄ + 0.6 cc. 1 <i>N</i> NaOH + 4 cc. H ₂ O.....	liquid
(21)	5 cc. 2% gel. + 0.2 cc. 1 <i>M</i> Na ₃ PO ₄ + 0.8 cc. 1 <i>N</i> NaOH + 4 cc. H ₂ O.....	liquid
(22)	5 cc. 2% gel. + 1.0 cc. 1 <i>N</i> NaOH + 4 cc. H ₂ O.....	liquid

TABLE II.
Gelatin—Phosphoric acid through phosphates to sodium hydroxide.

Solution number.	Mixture.	Physical state.
(1)	5 cc. 2% gel. +5.0 cc. H ₂ O (control).....	solid
(2)	5 cc. 2% gel. +0.10 cc. 1 <i>N</i> H ₃ PO ₄ +4.90 cc. H ₂ O.....	liquid
(3)	5 cc. 2% gel. +0.08 cc. 1 <i>N</i> H ₃ PO ₄ +0.02 cc. 1 <i>M</i> NaH ₂ PO ₄ +4.9 cc. H ₂ O.....	liquid
(4)	5 cc. 2% gel. +0.06 cc. 1 <i>N</i> H ₃ PO ₄ +0.04 cc. 1 <i>M</i> NaH ₂ PO ₄ +4.9 cc. H ₂ O.....	semi-solid
(5)	5 cc. 2% gel. +0.04 cc. 1 <i>N</i> H ₃ PO ₄ +0.06 cc. 1 <i>M</i> NaH ₂ PO ₄ +4.9 cc. H ₂ O.....	semi-solid
(6)	5 cc. 2% gel. +0.02 cc. 1 <i>N</i> H ₃ PO ₄ +0.08 cc. 1 <i>M</i> NaH ₂ PO ₄ +4.9 cc. H ₂ O.....	solid
(7)	5 cc. 2% gel. +0.10 cc. 1 <i>M</i> NaH ₂ PO ₄ +4.90 cc. H ₂ O.....	solid
(8)	5 cc. 2% gel. +0.08 cc. 1 <i>M</i> NaH ₂ PO ₄ +0.02 cc. 1 <i>M</i> Na ₂ HPO ₄ +4.9 cc. H ₂ O.....	solid
(9)	5 cc. 2% gel. +0.06 cc. 1 <i>M</i> NaH ₂ PO ₄ +0.04 cc. 1 <i>M</i> Na ₂ HPO ₄ +4.9 cc. H ₂ O.....	solid
(10)	5 cc. 2% gel. +0.04 cc. 1 <i>M</i> NaH ₂ PO ₄ +0.06 cc. 1 <i>M</i> Na ₂ HPO ₄ +4.9 cc. H ₂ O.....	solid
(11)	5 cc. 2% gel. +0.02 cc. 1 <i>M</i> NaH ₂ PO ₄ +0.08 cc. 1 <i>M</i> Na ₂ HPO ₄ +4.9 cc. H ₂ O.....	solid
(12)	5 cc. 2% gel. +0.10 cc. 1 <i>M</i> Na ₂ HPO ₄ +4.90 cc. H ₂ O.....	solid
(13)	5 cc. 2% gel. +0.08 cc. 1 <i>M</i> Na ₂ HPO ₄ +0.02 cc. 1 <i>M</i> Na ₃ PO ₄ +4.9 cc. H ₂ O.....	solid
(14)	5 cc. 2% gel. +0.06 cc. 1 <i>M</i> Na ₂ HPO ₄ +0.04 cc. 1 <i>M</i> Na ₃ PO ₄ +4.9 cc. H ₂ O.....	solid
(15)	5 cc. 2% gel. +0.04 cc. 1 <i>M</i> Na ₂ HPO ₄ +0.06 cc. 1 <i>M</i> Na ₃ PO ₄ +4.9 cc. H ₂ O.....	semi-solid
(16)	5 cc. 2% gel. +0.02 cc. 1 <i>M</i> Na ₂ HPO ₄ +0.08 cc. 1 <i>M</i> Na ₃ PO ₄ +4.9 cc. H ₂ O.....	semi-solid
(17)	5 cc. 2% gel. +0.10 cc. 1 <i>M</i> Na ₃ PO ₄ +4.90 cc. H ₂ O.....	semi-solid
(18)	5 cc. 2% gel. +0.08 cc. 1 <i>M</i> Na ₃ PO ₄ +0.02 cc. 1 <i>N</i> NaOH +4.9 cc. H ₂ O.....	liquid
(19)	5 cc. 2% gel. +0.06 cc. 1 <i>M</i> Na ₃ PO ₄ +0.04 cc. 1 <i>N</i> NaOH +4.9 cc. H ₂ O.....	liquid
(20)	5 cc. 2% gel. +0.04 cc. 1 <i>M</i> Na ₃ PO ₄ +0.06 cc. 1 <i>N</i> NaOH +4.9 cc. H ₂ O.....	liquid
(21)	5 cc. 2% gel. +0.02 cc. 1 <i>M</i> Na ₃ PO ₄ +0.08 cc. 1 <i>N</i> NaOH +4.9 cc. H ₂ O.....	liquid
(22)	5 cc. 2% gel. +0.10 cc. 1 <i>N</i> NaOH +4.9 cc. H ₂ O.....	liquid

however, as shown in Table II, identical with those previously described in connection with Table I.

As an example of another polybasic acid commonly found in living protoplasm, we chose citric acid, studying its effects by noting the results incident to progressive change from citric acid through mono-, di- and trisodium citrate in equimolar concentrations to pure sodium hydroxide.

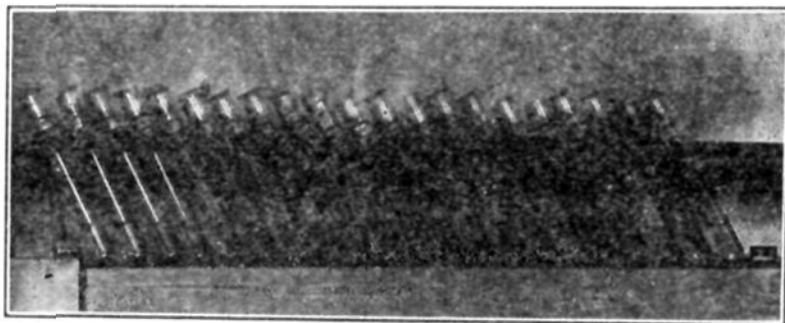


Fig. 2.

As shown in Table III, and Fig. 2, gelatin again remains solid in the middle of such a series and tends to liquefy as we pass toward the acid or alkaline extreme.

In Table IV and Fig. 3 are shown the effects of progressive change from sodium bicarbonate through sodium carbonate to sodium hydroxide. The carbonates in the concentrations indicated in this table showed a greater tendency to liquefy the gelatin than was observed in the phosphate

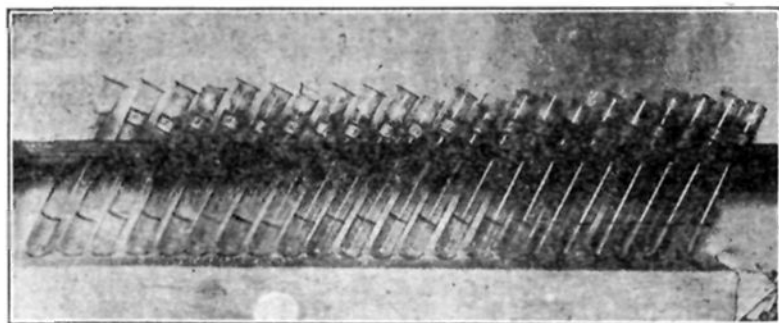


Fig. 3.

or citrate mixtures previously discussed. To show the effects of the different salt mixtures, this series of experiments was therefore kept at a somewhat lower temperature, namely, 5° C. At this temperature the gelatin remains solid in the pure sodium bicarbonate but tends to liquefy as this is replaced by carbonate or the carbonate by pure sodium hydroxide.

III. Remarks.

These experiments bring further proof that the hydration and the "solution" of a protein are not the same thing. Were solution merely the continuation of hydration then a liquid gelatin near the gelatin point

TABLE III.

Gelatin—Citric acid through citrates to sodium hydroxide.

Solution number.	Mixture.	Physical state.
(X)	5 cc. 2% gel. +5.0 cc. H ₂ O (control).....	solid
(1)	5 cc. 2% gel. +1.0 cc. 1 <i>N</i> citric acid +4.0 cc. H ₂ O.....	liquid
(2)	5 cc. 2% gel. +0.8 cc. 1 <i>N</i> citric acid +0.8 cc. 0.25 <i>M</i> monosod. cit. +3.4 cc. H ₂ O.....	semi-solid
(3)	5 cc. 2% gel. +0.6 cc. 1 <i>N</i> citric acid +1.6 cc. 0.25 <i>M</i> monosod. cit. +2.8 cc. H ₂ O.....	solid
(4)	5 cc. 2% gel. +0.4 cc. 1 <i>N</i> citric acid +2.4 cc. 0.25 <i>M</i> monosod. cit. +2.2 cc. H ₂ O.....	solid
(5)	5 cc. 2% gel. +0.2 cc. 1 <i>N</i> citric acid +3.2 cc. 0.25 <i>M</i> monosod. cit. +1.6 cc. H ₂ O.....	solid
(6)	5 cc. 2% gel. +4.0 cc. 0.25 <i>M</i> monosod. cit. +1.0 cc. H ₂ O.....	solid
(7)	5 cc. 2% gel. +3.2 cc. 0.25 <i>M</i> monosod. cit. +0.8 cc. 0.25 <i>M</i> disod. cit. +1.0 cc. H ₂ O.....	solid
(8)	5 cc. 2% gel. +2.4 cc. 0.25 <i>M</i> monosod. cit. +1.6 cc. 0.25 <i>M</i> disod. cit. +1.0 cc. H ₂ O.....	solid
(9)	5 cc. 2% gel. +1.6 cc. 0.25 <i>M</i> monosod. cit. +2.4 cc. 0.25 <i>M</i> disod. cit. +1.0 cc. H ₂ O.....	solid
(10)	5 cc. 2% gel. +0.8 cc. 0.25 <i>M</i> monosod. cit. +3.2 cc. 0.25 <i>M</i> disod. cit. +1.0 cc. H ₂ O.....	solid
(11)	5 cc. 2% gel. +4.0 cc. 0.25 <i>M</i> disod. cit. +1.0 cc. H ₂ O.....	solid
(12)	5 cc. 2% gel. +3.2 cc. 0.25 <i>M</i> disod. cit. +0.8 cc. 0.25 <i>M</i> trisod. cit. +1.0 cc. H ₂ O.....	solid
(13)	5 cc. 2% gel. +2.4 cc. 0.25 <i>M</i> disod. cit. +1.6 cc. 0.25 <i>M</i> trisod. cit. +1.0 cc. H ₂ O.....	solid
(14)	5 cc. 2% gel. +1.6 cc. 0.25 <i>M</i> disod. cit. +2.4 cc. 0.25 <i>M</i> trisod. cit. +1.0 cc. H ₂ O.....	solid
(15)	5 cc. 2% gel. +0.8 cc. 0.25 <i>M</i> disod. cit. +3.2 cc. 0.25 <i>M</i> trisod. cit. +1.0 cc. H ₂ O.....	solid
(16)	5 cc. 2% gel. +4.0 cc. 0.25 <i>M</i> trisod. cit. +1.0 cc. H ₂ O.....	solid
(17)	5 cc. 2% gel. +3.2 cc. 0.25 <i>M</i> trisod. cit. +0.8 cc. 1 <i>N</i> NaOH +1.0 cc. H ₂ O.....	liquid
(18)	5 cc. 2% gel. +2.4 cc. 0.25 <i>M</i> trisod. cit. +1.6 cc. 1 <i>N</i> NaOH +1.0 cc. H ₂ O.....	liquid
(19)	5 cc. 2% gel. +1.6 cc. 0.25 <i>M</i> trisod. cit. +2.4 cc. 1 <i>N</i> NaOH +1.0 cc. H ₂ O.....	liquid
(20)	5 cc. 2% gel. +0.8 cc. 0.25 <i>M</i> trisod. cit. +3.2 cc. 1 <i>N</i> NaOH +1.0 cc. H ₂ O.....	liquid
(21)	5 cc. 2% gel. +4.0 cc. 1 <i>N</i> NaOH +1.0 cc. H ₂ O.....	liquid

TABLE IV.
Gelatin—Sodium bicarbonate through sodium carbonate to sodium hydroxide.

Solution number.	Mixture.	Physical state.
(X)	5 cc. 2% gel. +5.00 cc. H ₂ O (control).....	solid
(1)	5 cc. 2% gel. +0.20 cc. 1 <i>M</i> NaHCO ₃ +4.80 cc. H ₂ O.....	solid
(2)	5 cc. 2% gel. +0.18 cc. 1 <i>M</i> NaHCO ₃ +0.02 cc. 1 <i>M</i> Na ₂ CO ₃ +4.8 cc. H ₂ O.....	solid
(3)	5 cc. 2% gel. +0.16 cc. 1 <i>M</i> NaHCO ₃ +0.04 cc. 1 <i>M</i> Na ₂ CO ₃ +4.8 cc. H ₂ O.....	solid
(4)	5 cc. 2% gel. +0.14 cc. 1 <i>M</i> NaHCO ₃ +0.06 cc. 1 <i>M</i> Na ₂ CO ₃ +4.8 cc. H ₂ O.....	solid
(5)	5 cc. 2% gel. +0.12 cc. 1 <i>M</i> NaHCO ₃ +0.08 cc. 1 <i>M</i> Na ₂ CO ₃ +4.8 cc. H ₂ O.....	solid
(6)	5 cc. 2% gel. +0.10 cc. 1 <i>M</i> NaHCO ₃ +0.10 cc. 1 <i>M</i> Na ₂ CO ₃ +4.8 cc. H ₂ O.....	solid
(7)	5 cc. 2% gel. +0.08 cc. 1 <i>M</i> NaHCO ₃ +0.12 cc. 1 <i>M</i> Na ₂ CO ₃ +4.8 cc. H ₂ O.....	solid
(8)	5 cc. 2% gel. +0.06 cc. 1 <i>M</i> NaHCO ₃ +0.14 cc. 1 <i>M</i> Na ₂ CO ₃ +4.8 cc. H ₂ O.....	solid
(9)	5 cc. 2% gel. +0.04 cc. 1 <i>M</i> NaHCO ₃ +0.16 cc. 1 <i>M</i> Na ₂ CO ₃ +4.8 cc. H ₂ O.....	solid
(10)	5 cc. 2% gel. +0.02 cc. 1 <i>M</i> NaHCO ₃ +0.18 cc. 1 <i>M</i> Na ₂ CO ₃ +4.8 cc. H ₂ O.....	solid
(11)	5 cc. 2% gel. +0.20 cc. 1 <i>M</i> Na ₂ CO ₃ +4.80 cc. H ₂ O.....	solid
(12)	5 cc. 2% gel. +0.18 cc. 1 <i>M</i> Na ₂ CO ₃ +0.02 cc. 1 <i>N</i> NaOH +4.8 cc. H ₂ O.....	solid
(13)	5 cc. 2% gel. +0.16 cc. 1 <i>M</i> Na ₂ CO ₃ +0.04 cc. 1 <i>N</i> NaOH +4.8 cc. H ₂ O.....	solid
(14)	5 cc. 2% gel. +0.14 cc. 1 <i>M</i> Na ₂ CO ₃ +0.06 cc. 1 <i>N</i> NaOH +4.8 cc. H ₂ O.....	semi-solid
(15)	5 cc. 2% gel. +0.12 cc. 1 <i>M</i> Na ₂ CO ₃ +0.08 cc. 1 <i>N</i> NaOH +4.8 cc. H ₂ O.....	semi-solid
(16)	5 cc. 2% gel. +0.10 cc. 1 <i>M</i> Na ₂ CO ₃ +0.10 cc. 1 <i>N</i> NaOH +4.8 cc. H ₂ O.....	liquid
(17)	5 cc. 2% gel. +0.08 cc. 1 <i>M</i> Na ₂ CO ₃ +0.12 cc. 1 <i>N</i> NaOH +4.8 cc. H ₂ O.....	liquid
(18)	5 cc. 2% gel. +0.06 cc. 1 <i>M</i> Na ₂ CO ₃ +0.14 cc. 1 <i>N</i> NaOH +4.8 cc. H ₂ O.....	liquid
(19)	5 cc. 2% gel. +0.04 cc. 1 <i>M</i> Na ₂ CO ₃ +0.16 cc. 1 <i>N</i> NaOH +4.8 cc. H ₂ O.....	liquid
(20)	5 cc. 2% gel. +0.02 cc. 1 <i>M</i> Na ₂ CO ₃ +0.18 cc. 1 <i>N</i> NaOH +4.8 cc. H ₂ O.....	liquid
(21)	5 cc. 2% gel. +0.20 cc. 1 <i>N</i> NaOH +4.80 cc. H ₂ O.....	liquid

should be found to gel upon the addition of acid, for the addition of acid increases hydration. As our previous experiments have shown and as indicated by those detailed in this paper, opposite conditions obtain, for the addition of acid or of alkali will make an already solid gelatin gel become not stiffer but more liquid. We seem to be justified in the assumption that gelatin is a chemical substance capable of existing in different degrees of association or polymerization depending upon the temperature and upon other changes in its environment, like the presence of acids or of alkalies. The degree of association and hence the size of the particles of which the gelatin is composed may be greatly varied. At the higher temperatures and under the influences of acids and alkalies, for example, the particles become smaller, while under reverse conditions they become larger. With these changes in size, they change their physicochemical properties so that under the former circumstances they are liquid and clear, while under the latter they become solid and opalescent. The particles seem capable of absorbing most water (becoming most heavily hydrated) when they have a medium diameter. Neutral gelatin in which the particles are large, therefore absorbs some water, which absorption on the addition of acid (which multiplies the particles and makes them smaller) is increased. On further addition of acid, however, the particles decrease in size to beyond that optimal for swelling. In this region the mixture as a whole begins to liquefy and shortly thereafter to show prominent evidences of "going into solution."

These experiments seem to us to help to explain the successive changes observable in living cells when these are subjected to a direct acid intoxication or to an indirect acid intoxication through so-called "injury" by mechanical, thermal or chemical means. After injury, tissues pass through a primary period of swelling into a second one of softening¹ with evidences of protoplasmic liquefaction and solution. In the terms of colloid chemistry, we would say that under the influences of the acids and similarly acting substances brought into play through the injury, the tissues first swell; as their acid content rises, protein dissociation becomes more prominent and betrays itself by a greater tendency of the tissues to liquefy and since hydration is now less, the tissues soften and "dissolve" in the media bathing them (be this sea water, blood, lymph or urine).

These experiments also bear upon the problem of digestion and that special phase of it known as autolysis. The first changes observable in these reactions consist of swelling, followed by a softening and dissolution of the proteins acted upon. Acids and alkalies have long been

¹ For a discussion of the "solidity" of living tissues as dependent upon their emulsion character and for the "softening" of such tissues consequent upon the "breaking" of the emulsion see Martin H. Fischer and Marion O. Hooker, "Fats and Fatty Degeneration," New York, 76 (1917).

known to favor these initial steps in proteolysis, while all salts, including such "buffer" salts as phosphates or citrates, have been known to inhibit them. Their action has usually been laid to an effect upon the enzymes themselves. As has been pointed out before, acids, alkalies and salts produce at least as large and probably their greatest effects upon the proteins undergoing digestion. The important theoretical and practical bearings of such considerations upon laboratory practice and the everyday problems of the hanging of meat, its preservation by salting, the prevention of putrefaction, etc., are self-evident.

These experiments emphasize again the necessity of interpreting in the simpler language of colloid-chemistry the mass of experimental material now collected by the biologists under the heading of "permeability" studies. It means little to say that under the influence of acids or of substances which in living cells produce acid effects (like the anesthetics) the "permeability" of the "plasma" membranes surrounding cells is increased so that albumin gets out or salts get in. Not only are plasma membranes figments of the imagination, but nothing is gained by heaping "permeability" properties upon them. "Permeability" is a physiological concept which itself needs to be explained. The proteins throughout a cell (not only in its hypothetical overcoat) can under the influence of acids, for example, be made to absorb water, to absorb salt,¹ to soften and to give off albumin. And as all these effects may be observed in simple proteins where their exact nature is more accessible to quantitative study and further analysis than in the case of our complex living cells, it would look like a better conservation of mental energy to concentrate further study upon the colloid-chemical principles here to the front than to continue indefinitely the mere restatement of biological problems in the terms of biology itself.

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THE UTILIZATION OF THE ADSORPTIVE POWER OF FULLER'S EARTH FOR CHEMICAL SEPARATIONS.

By ATHERTON SEIDELL.

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The utilization of fuller's earth for chemical separations (other than decolorizations) has, in recent years, found at least two practical applications. The first of these is its use in connection with the isolation of alkaloids² from plants or their extracts and the second forms an important

¹ Martin H. Fischer, *J. Am. Med. Assoc.*, 64, 325 (1915).

² Sigmund Waldbott, "Precipitating Alkaloids by Lloyd's Reagent—Preliminary Note," *THIS JOURNAL*, 35, 837 (1913).