#### Summary.

1. Experiments are described showing the amounts of water absorbed by gelatin discs immersed in different concentrations of the primary, binary or ternary salts of phosphoric, citric and carbonic acids. The swelling varies not only with the salt but with its concentration.

2. In further experiments are detailed the absorption of water in phosphate and citrate mixtures varying from the extreme of the pure acid on the one side through the mono-, di- and trisodium salts of these acids to pure sodium hydroxide on the other. Irrespective of the manner in which these 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 absorbed is plotted on the vertical and change in composition of the mixture on the horizontal, that the result yields a V-shaped or U-shaped curve. From a minimal point in the middle of this curve, there is a progressive increase in water absorption to the left or to the right as acid content or alkali content of the mixture is increased.

3. What has been said of phosphate and citrate mixtures holds also for carbonate mixtures.

4. 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.

CINCINNATI, OHIO.

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

# ON THE SWELLING OF FIBRIN IN POLYBASIC ACIDS AND THEIR SALTS.

BY MARTIN H. FISCHER AND MARTIN BENZINGER.

Received November 2, 1917.

#### I.

It was shown in a previous paper<sup>1</sup> that water absorption by such a protein as gelatin increases even in the presence of so-called buffer salts with every increase in the acid or alkali content of the mixture on either side of a low point. This conclusion is contrary to much generally accepted opinion.<sup>2</sup> In order to show that the behavior of gelatin is not exceptional

 $^1$  Martin H. Fischer and Marian O. Hooker, Science, 46, 189 (1917); cf. preceding article.

<sup>2</sup> See Max Koppel, *Deut. Arch. klin. Med.*, 112, 594 (1913); see also Henderson, Palmer and Newburgh, *J. Pharm. Exp. Therap.*, 5, 449 (1914) as well as their numerous followers.

the experiments detailed in this paper were undertaken. They show that the same general law holds for the absorption of water by another protein, namely fibrin. The fibrin was a preparation carefully prepared from blood and thoroughly washed to remove as many adhering salts as possible. After being dried at a low temperature it was pulverized in a mortar. Weighed amounts of the powder (0.5 g.) were then introduced into definite volumes (20 cc.) of the various solutions employed, contained in calibrated test tubes of uniform diameter (1.5 cm.). The same standard solutions of acids, alkali and of salts as were employed in the previous study were used in this one. All the mixtures in the various tubes were treated in exactly the same fashion, as to shaking, settling, etc. The height of the swollen fibrin columns, at the end of 24 hours, was taken as the index of water absorption. The results of our several series of experiments may be summed up as follows: II.

(a) We tested first the effects of a progressive change from monosodium citrate through disodium citrate to trisodium citrate in equimolar concentrations, continuing the series toward a pure acid on the one side and toward a pure alkali upon the other side, as shown in Table I.

As indicated by the heights of the fibrin columns, and better by the curve of Fig. 1 which expresses the results of this experiment graphically, greatest swelling is observed in the pure solutions of acid or alkali. From

these extremes the amount of swelling decreases as neutralization progresses until a low point is reached in the middle of the curve. This low point is observed in a mixture of, approximately, one molar equivalent of monosodium citrate with two molar equivalents of disodium citrate. In comparing this minimal point for fibrin with that obtained in the case of gelatin it is seen that in the latter instance it lies closer to the (theoretically) pure solution of monosodium citrate.

(b) In a next series of experiments we tested the effects of a gradual increase in the phosphoric acid content



### TABLE 1.

# Fibrin—Citric Acid through Citrates to Sodium Hydroxide.

Solution number.	Concentration of solution.	column in mm. after 24 hours.
I	2 cc. 1 N citric acid +18 cc. H <sub>2</sub> O	31.0
2	1.6 cc. 1 N citric acid +0.4 cc. 1 M monosodium citrate +18 cc. H <sub>2</sub> O	21.5
3	1.2 cc. 1 N citric acid +0.8 cc. 1 M monosodium citrate +18 cc. H <sub>2</sub> O	16.5
4	0.8 cc. 1 N citric acid +1.2 cc. 1 M monosodium citrate +18 cc. H <sub>2</sub> O	15.0
5	0.4 cc. I N citric acid +1.6 cc. I M monosodium citrate +18 cc. H <sub>2</sub> O	14.0
6	2 cc. I <i>M</i> monosodium citrate + 18 cc. H <sub>2</sub> O	14.0
7	1.6 cc 1 <i>M</i> monosodium citrate +0.4 cc. 1 <i>M</i> disodium citrate +18 cc. H <sub>2</sub> O	13.5
8	1.2 cc. 1 <i>M</i> monosodium citrate +0.8 cc. 1 <i>M</i> disodium citrate +18 cc. H <sub>2</sub> O	13.5
9	0.8 cc. I <i>M</i> monosodium citrate +1.2 cc. I <i>M</i> disodium citrate +18 cc. H <sub>2</sub> O	13.5
ю	0.4 cc. 1 <i>M</i> monosodium citrate +1.6 cc. 1 <i>M</i> disodium citrate +18 cc. H <sub>2</sub> O	13.0
II	2 cc. 1 <i>M</i> disodium citrate +18 cc. H <sub>2</sub> O	12.5
12	1.6 cc. 1 <i>M</i> disodium citrate +0.4 cc. 1 <i>M</i> trisodium citrate +18 cc. H <sub>2</sub> O	12.5
13	1.2 cc. 1 <i>M</i> disodium citrate +0.8 cc. 1 <i>M</i> trisodium citrate +18 cc. H <sub>2</sub> O	13.0
14	0.8 cc. 1 <i>M</i> disodium citrate +1.2 cc. 1 <i>M</i> trisodium citrate +18 cc. H <sub>2</sub> O	13.5
15	0.4 cc. 1 <i>M</i> disodium citrate +1.6 cc. 1 <i>M</i> trisodium citrate +18 cc. H <sub>2</sub> O	17.0
16	2 cc. 1 <i>M</i> trisodium citrate +18 cc. H <sub>2</sub> O	21.5
17	1.6 cc. 1 $M$ trisodium citrate +0.4 cc. 1 $N$ NaOH +18 cc. H <sub>2</sub> O	31.0
18	$1.2 \text{ cc. } 1  M \text{ trisodium citrate } +0.8 \text{ cc. } 1  N \text{ NaOH } +18 \text{ cc. } H_2 \text{ O}$	47.0
19	0.8 cc. 1 $M$ trisodium citrate +1.2 cc. 1 $N$ NaOH +18 cc. H <sub>2</sub> O	58.0
20	0.4 cc. I $M$ trisodium citrate + 1.6 cc. I $N$ NaOH + 18 cc. H <sub>2</sub> O	73.0
21	2 cc. 1 N laOH +18 cc. $H_2O$	96.5
22	20 cc. water (control)	12.5

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TABLE	II.
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Fibrin—Disodium Phosphate + Increasing Amounts of Phosphoric Acid.

		Height of fibrin
Solution number.	Concentration of solution.	column in mm. atter 24 hours.
I	$0.8 \text{ cc. } 0.25 M \text{ Na}_2\text{HPO}_4 + 0.44 \text{ cc. } 1 N \text{ H}_3\text{PO}_4 + 18.76 \text{ cc. } \text{H}_2\text{O}_2$	20.0
2	$0.8 \text{ cc. } 0.25 M \text{ Na}_2\text{HPO}_4 + 0.42 \text{ cc. } 1 N \text{ H}_3\text{PO}_4 + 18.78 \text{ cc. } \text{H}_2\text{O}$	19.0
3	$0.8 \text{ cc. } 0.25 M \text{ Na}_2\text{HPO}_4 + 0.40 \text{ cc. } 1 N \text{ H}_3\text{PO}_4 + 18.80 \text{ cc. } \text{H}_2\text{O}_2$	18.0
4	$0.8 \text{ cc. } 0.25 M \text{ Na}_2\text{HPO}_4 + 0.38 \text{ cc. } 1 N \text{ H}_3\text{PO}_4 + 18.82 \text{ cc. } \text{H}_2\text{O}_2$	18.0
5	$0.8 \text{ cc. } 0.25 M \text{ Na}_2\text{HPO}_4 + 0.36 \text{ cc. } 1 N \text{ H}_3\text{PO}_4 + 18.84 \text{ cc. } \text{H}_2\text{O}_2$	18.0
6	$0.8 \text{ cc. } 0.25 M \text{ Na}_2\text{HPO}_4 + 0.34 \text{ cc. } 1 N \text{ H}_3\text{PO}_4 + 18.86 \text{ cc. } \text{H}_2\text{O}_2$	17.5
7	$0.8 \text{ cc. } 0.25  M \text{ Na}_2\text{HPO}_4 + 0.32 \text{ cc. } 1  N \text{ H}_3\text{PO}_4 + 18.88 \text{ cc. } \text{H}_2\text{O}$	17.0
8	$0.8 \text{ cc. } 0.25 M \text{ Na}_2\text{HPO}_4 + 0.30 \text{ cc. } 1 N \text{ H}_3\text{PO}_4 + 18.90 \text{ cc. } \text{H}_2\text{O}_2$	16.5
9	$0.8 \text{ cc. } 0.25 M \text{ Na}_{2}\text{HPO}_{4} + 0.28 \text{ cc. } 1 N \text{ H}_{3}\text{PO}_{4} + 18.92 \text{ cc. } \text{H}_{2}\text{O}_{1}$	15.5
ю	$0.8 \text{ cc. } 0.25 M \text{ Na}_2\text{HPO}_4 + 0.26 \text{ cc. } 1 N \text{ H}_3\text{PO}_4 + 18.94 \text{ cc. } \text{H}_2\text{O}_2\text{ cc. } 1 \text{ N}_2\text{O}_2\text{ cc. } 1 \text{ N}_2\text{O}_$	15.0
II	$0.8 \text{ cc. } 0.25 M \text{ Na}_2\text{HPO}_4 + 0.24 \text{ cc. } 1 N \text{ H}_2\text{PO}_4 + 18.96 \text{ cc. } \text{H}_2\text{O}_2\text{ cc. } 1 \text{ N}_2\text{O}_2\text{ cc. } 1 \text{ N}_2\text{ cc. } 1 \text{ Cc. } 1  Cc. $	14.5
12	$0.8 \text{ cc. } 0.25  M \text{ Na}_2\text{HPO}_4 + 0.22 \text{ cc. } 1  N \text{ H}_2\text{PO}_4 + 18.98 \text{ cc. } \text{H}_2\text{O}$	14.0
13	$0.8 \text{ cc. } 0.25 M \text{ Na}_2\text{HPO}_4 + 0.20 \text{ cc. } 1 N \text{ H}_2\text{PO}_4 + 19.00 \text{ cc. } \text{H}_2\text{O}_2\text{ cc. } 1 \text{ Cc. } 1  Cc. $	14.0
14	$0.8 \text{ cc. } 0.25  M \text{ Na}_2\text{HPO}_4 + 0.18 \text{ cc. } 1  N \text{ H}_2\text{PO}_4 + 19.02 \text{ cc. } \text{H}_2\text{O}_2 \text{ cc. } \text{Cc. } \text{H}_2 \text{O}_2 \text{ cc. } \text{Cc. } \text{H}_2 \text{O}_2 \text{ cc. } \text{Cc. } \text{H}_2 \text{ cc. } \text{Cc. } C$	14.0
15	$0.8 \text{ cc. } 0.25 M \text{ Na}_2\text{HPO}_4 + 0.16 \text{ cc. } 1 N \text{ H}_2\text{PO}_4 + 19.04 \text{ cc. } \text{H}_2\text{O}_2\text{ cc. } $	13.5
16	$0.8 \text{ cc. } 0.25  M \text{ Na}_2\text{HPO}_4 + 0.14 \text{ cc. } 1  N \text{ H}_2\text{PO}_4 + 19.06 \text{ cc. } \text{H}_2\text{O}_2 \dots $	14.0
17	$0.8 \text{ cc. } 0.25 M \text{ Na}_2\text{HPO}_4 + 0.12 \text{ cc. } 1 N \text{ H}_2\text{PO}_4 + 19.08 \text{ cc. } \text{H}_2\text{O}_2$	14.0
18	$0.8 \text{ cc. } 0.25 M \text{ Na}_2\text{HPO}_4 + 0.10 \text{ cc. } 1 N \text{ H}_2\text{PO}_4 + 19.10 \text{ cc. } \text{H}_2\text{O}_2$	14.5
19	$0.8 \text{ cc. } 0.25 M \text{ Na}_2\text{HPO}_4 + 0.08 \text{ cc. } 1 N \text{ H}_2\text{PO}_4 + 19.12 \text{ cc. } \text{H}_2\text{O}_2$	14.5
20	$0.8 \text{ cc. } 0.25 M \text{ Na}_2\text{HPO}_4 + 0.06 \text{ cc. } 1 N \text{ H}_4\text{PO}_4 + 19.14 \text{ cc. } \text{H}_2\text{O}$	14.5
2 I	$0.8 \text{ cc. } 0.25  M \text{ Na}_2\text{HPO}_4 + 0.04 \text{ cc. } 1  N \text{ H}_2\text{PO}_4 + 19.16 \text{ cc. } \text{H}_2\text{O}_2$	14.5
22	$0.8 \text{ cc. } 0.25  M \text{ Na}_2\text{HPO}_4 + 0.02 \text{ cc. } 1  N \text{ H}_2\text{PO}_4 + 19.18 \text{ cc. } \text{H}_2\text{O}$	15.5
23	$0.8 \text{ cc. } 0.25 M \text{ Na}_2\text{HPO}_4 + 19.2 \text{ cc. } H_2\text{O}_2$	16.0
24	20 cc. water (control)	12.5

of a solution containing a fixed amount of disodium phosphate. The results are shown in Table II and Fig. 2. When phosphoric acid is added



to disodium phosphate there is at first a decrease in the amount of swelling. This gives way later to an increased swelling as the concentration of the acid passes a certain point. The experi-

mental results show that this point of minimal swelling is observed in a mixture which theoretically is composed of about molar equivalents of

disodium phosphate and monosodium phosphate.

(c) In Table III and Fig. 3 are shown the effects of adding to the same concentration of disodium phosphate used in the previous experiment, progressively greater amounts of sodium hydroxide. There is, with every increase in the concentration of the added alkali, an increase in the height of the swelling column.

(d) The effects of adding progressively greater amounts of sodium hydroxide to a fixed amount of monosodium phosphate of the same molar concentration as the disodium phosphate used in the previous experiments are shown in Table IV and Fig. 4.



When small amounts of the alkali are added to the monosodium phosphate there is to be observed at first a decrease in the amount of swelling which later, however, with further increase in the amount of alkali added, gives way to an increased swelling. It is again obvious that the low point in the swelling curve is found at a point at which the mixture is essentially one of molar equivalents of monosodium and disodium phosphate.

(e) The results of another type of variations in experimental procedure is shown in Table V and Fig. 5. Here a fixed amount of phosphoric acid has added to it progressively greater amounts of sodium hydroxide. This arrangement allows us to see the effects of simultaneously reducing acid content while increasing the amount and kind of phosphate present.

TABLE III. Fibrin—Disodium Phosphate + Increasing Amounts of Sodium Hydroxide.

Solution number.	Concentration of solution.	Height of fibrin column in mm. after 24 hours.
I	$0.8 \text{ cc. } 0.25 M \text{ Na}_2\text{HPO}_4 + 19.2 \text{ cc. } H_2\text{O}$	16.0
2	$0.8 \text{ cc. } 0.25 M \text{ Na}_2\text{HPO}_4 + 0.02 \text{ cc. } 1 N \text{ NaOH} + 19.18 \text{ cc. } H_2\text{O}$	16.5
3	$0.8 \text{ cc. } 0.25  M \text{ Na}_2\text{HPO}_4 + 0.04 \text{ cc. } 1  N \text{ AOH} + 19.16 \text{ cc. } H_2\text{O} \dots$	17.5
4	$0.8 \text{ cc. } 0.25 M \text{ Na}_2\text{HPO}_4 + 0.06 \text{ cc. } 1 N \text{ NaOH} + 19.14 \text{ cc. } H_2\text{O}$	18.5
5	$0.8 \text{ cc. } 0.25 M \text{ Na}_2\text{HPO}_4 + 0.08 \text{ cc. } 1 N \text{ NaOH} + 19.12 \text{ cc. } H_2\text{O}$	19.5
6	$0.8 \text{ cc. } 0.25 M \text{ Na}_2\text{HPO}_4 + 0.10 \text{ cc. } 1 N \text{ NaOH} + 19.10 \text{ cc. } H_2\text{O}$	21.0
7	$0.8 \text{ cc. } 0.25 M \text{ Na}_2\text{HPO}_4 + 0.12 \text{ cc. } 1 N \text{ NaOH} + 19.08 \text{ cc. } H_2\text{O}$	22.0
8	$0.8 \text{ cc. } 0.25 M \text{ Na}_2\text{HPO}_4 + 0.14 \text{ cc. } 1 N \text{ NaOH} + 19.06 \text{ cc. } H_2\text{O}$	23.0
9	$0.8 \text{ cc. } 0.25 M \text{ Na}_2\text{HPO}_4 + 0.16 \text{ cc. } 1 N \text{ NaOH} + 19.04 \text{ cc. } H_2\text{O}$	24.5
IO	$0.8 \text{ cc. } 0.25 M \text{ Na}_2\text{HPO}_4 + 0.18 \text{ cc. } 1 N \text{ NaOH} + 19.02 \text{ cc. } H_2\text{O}$	26.5
II	$0.8 \text{ cc. } 0.25 M \text{ Na}_2\text{HPO}_4 + 0.20 \text{ cc. } 1 N \text{ NaOH} + 19.00 \text{ cc. } H_2\text{O}$	29.5
I 2	$0.8 \text{ cc. } 0.25 M \text{ Na}_2\text{HPO}_4 + 0.22 \text{ cc. } 1 N \text{ NaOH} + 18.98 \text{ cc. } H_2\text{O}$	•• 34.5
13	20 cc. water (control)	12.5

#### TABLE IV.

# Fibrin-Monosodium Phosphate + Increasing Amounts of Sodium Hydroxide.

		TT-1-1-4
Solution number.	Concentration of solution.	column in mm. alter 24 hours.
I	0.2 cc. 1 <i>M</i> NaH <sub>2</sub> PO <sub>4</sub> +19.08 cc. H <sub>2</sub> O	16.0
2	0.2 cc. 1 M NaH <sub>2</sub> PO <sub>4</sub> + 0.02 cc. 1 N NaOH +19.78 cc. H <sub>2</sub> O	15.5
3	0.2 cc. 1 M NaH <sub>2</sub> PO <sub>4</sub> + 0.04 cc. 1 N NaOH + 19.76 cc. H <sub>2</sub> O	15.5
4	0.2 cc. 1 M NaH <sub>2</sub> PO <sub>4</sub> + 0.06 cc. 1 N NaOH + 19.74 cc. H <sub>2</sub> O	15.5
5	$0.2 \text{ cc. } I M \text{ NaH}_2\text{PO}_4 + 0.08 \text{ cc. } I N \text{ NaOH} + 19.72 \text{ cc. } H_2\text{O}$	15.0
6	0.2 cc. 1 M NaH <sub>2</sub> PO <sub>4</sub> + 0.10 cc. 1 N NaOH + 19.70 cc. H <sub>2</sub> O	15.0
7	0.2 cc. 1 M NaH <sub>2</sub> PO <sub>4</sub> + 0.12 cc. 1 N NaOH +19.68 cc. H <sub>2</sub> O	14.0
8	0.2 cc. 1 <i>M</i> NaH <sub>2</sub> PO <sub>4</sub> + 0.14 cc. 1 <i>N</i> NaOH + 19.66 cc. H <sub>2</sub> O.	15.0
9	$0.2 \text{ cc. } I M \text{ NaH}_2\text{PO}_4 + 0.16 \text{ cc. } I N \text{ NaOH} + 19.64 \text{ cc. } H_2\text{O}$	15.5
10	0.2 cc. 1 <i>M</i> NaH <sub>2</sub> PO <sub>4</sub> + 0.18 cc. 1 <i>N</i> NaOH +19.62 cc. H <sub>2</sub> O	15.5
II	$0.2 \text{ cc. } 1 M \text{ NaH}_2\text{PO}_4 + 0.20 \text{ cc. } 1 N \text{ NaOH} + 19.60 \text{ cc. } \text{H}_2\text{O}_2\text{ cc. } 1 \text{ Cc. } \text{Cc. } \text{H}_2\text{O}_2\text{ cc. } \text{Cc. }$	15.5
12	0.2 cc. 1 M NaH <sub>2</sub> PO <sub>4</sub> + 0.22 cc. 1 N NaOH +19.58 cc. H <sub>2</sub> O	16.0
13	$0.2 \text{ cc. I } M \text{ NaH}_2\text{PO}_4 + 0.24 \text{ cc. I } N \text{ NaOH} + 19.56 \text{ cc. H}_2\text{O}_{$	16.5
14	0.2 cc. 1 M NaH <sub>2</sub> PO <sub>4</sub> + 0.26 cc. 1 N NaOH +19.54 cc. H <sub>2</sub> O	18.0
15	0.2 cc. 1 <i>M</i> NaH <sub>2</sub> PO <sub>4</sub> + $0.28$ cc. 1 <i>N</i> NaOH + 19.52 cc. H <sub>2</sub> O	19.5
16	0.2 cc. 1 M NaH <sub>2</sub> PO <sub>4</sub> + 0.30 cc. 1 N NaOH +19.50 cc. H <sub>2</sub> O	21.5
17	0.2 cč. 1 M NaH <sub>2</sub> PO <sub>4</sub> + 0.32 cc. 1 N NaOH +19.48 cc. H <sub>2</sub> O	23.5
18	$0.2 \text{ cc. I } M \text{ NaH}_{2}\text{PO}_{4} + 0.34 \text{ cc. I } N \text{ NaOH} + 19.46 \text{ cc. H}_{2}\text{O}_{2}$	25.0
19	$0.2 \text{ cc. I } M \text{ NaH}_2\text{PO}_4 + 0.36 \text{ cc. I } N \text{ NaOH} + 19.44 \text{ cc. H}_2\text{O}$	27.5
20	0.2 cc. 1 M NaH <sub>2</sub> PO <sub>4</sub> + 0.38 cc. 1 N NaOH + 19.42 cc. H <sub>2</sub> O	33.0
2 I	20 cc. water (control)	12.5

The result is again, however, a curve of the same general type already discussed. Beginning with the greatest swelling in the pure acid there is a gradual fall in the curve until a low point is reached in a mixture lying midway between the (theoretically) pure monosodium phosphate and the pure disodium phosphate. From this point represented by



the pure disodium phosphate the swelling then increases more abruptly as we enter the realm of the pure trisodium phosphate or this plus unneutralized alkali.



(f) A final type of variations in experimental procedure is shown in Table VI and Fig. 6. Here a fixed amount of alkali has added to it progressively greater amounts of phosphoric acid until neutralization is carried to the point of getting a (theoretically) pure solution of trisodium phosphate. The progressive decrease in the amount of swelling with decrease in alkalinity and increase in phosphate content is readily apparent.

TABLE V.		
Solution	Fibrin—Phosphoric Acid + Increasing Amounts of Sodium Hydroxide.	Height of fibrin column in mm.
number.	Concentration of solution.	after 24 hours.
I	$0.6 \text{ cc. } I N \text{ H}_3\text{PO}_4 + 0.02 \text{ cc. } I N \text{ NaOH} + 19.38 \text{ cc. } \text{H}_2\text{O}$	16.5
2	$0.6 \text{ cc. } 1 N \text{ H}_3 \text{PO}_4 + 0.04 \text{ cc. } 1 N \text{ NaOH} + 19.36 \text{ cc. } \text{H}_2 \text{O}_2 \text{ cc. } \text{H}_2 \text{Cc. } \text{H}_2 \text{O}_2 \text{ cc. } \text{H}_2 \text{O}_2 \text{ cc. } \text{H}_2 \text{Cc. } \text{H}_2 \text{Cc. } \text{H}_2 \text{Cc. } \text{Cc. } \text{Cc. } \text{H}_2 \text{Cc. } \text{Cc. } \text{H}_2 \text{Cc. } \text{Cc. } \text{Cc. } \text{H}_2 \text{Cc. } Cc. $	15.5
3	$0.6 \text{ cc. } 1 \text{ N} \text{ H}_{3}\text{PO}_{4} + 0.06 \text{ cc. } 1 \text{ N} \text{ NaOH} + 19.34 \text{ cc. } \text{H}_{2}\text{O}$	15.0
4	$0.6 \text{ cc. } 1 N \text{ H}_{4}\text{PO}_{4} + 0.08 \text{ cc. } 1 N \text{ aOH} + 19.32 \text{ cc. } \text{H}_{2}\text{O}$	14.5
5	$0.6 \text{ cc. } 1 \text{ N} \text{ H}_{4}\text{PO}_{4} + 0.10 \text{ cc. } 1 \text{ N} \text{ NaOH} + 19.30 \text{ cc. } \text{H}_{2}\text{O}$	14.0
6	$0.6 \text{ cc. I } N \text{ H}_{3}\text{PO}_{4} + 0.12 \text{ cc. I } N \text{ aOH } + 19.28 \text{ cc. H}_{2}\text{O}_{2}$	13.0
7	$0.6 \text{ cc. I } N \text{ H}_{3}PO_{4} + 0.14 \text{ cc. I } N \text{ a}OH + 19.26 \text{ cc. H}_{2}O$	12.5
8	$0.6 \text{ cc. } I N \text{H}_{3}\text{PO}_{4} + 0.16 \text{ cc. } I N \text{ a}\text{O}\text{H} + 19.24 \text{ cc. } \text{H}_{2}\text{O}$	12.5
9	0.6 cc. 1 N H <sub>3</sub> PO <sub>4</sub> +0.18 cc. 1 N NaOH +19.22 cc. H <sub>4</sub> O	12.5
ю	$0.6 \text{ cc. I } N \text{ H}_{3} PO_{4} + 0.20 \text{ cc. I } N \text{ NaOH } + 19.20 \text{ cc. H}_{2} O_{2} \dots \dots$	12.5
II	0.6 cc. 1 N H <sub>4</sub> PO <sub>4</sub> +0.22 cc. 1 N NaOH +19.18 cc. H <sub>2</sub> O	12.5
12	0.6 cc. 1 N H <sub>1</sub> PO <sub>4</sub> + 0.24 cc. 1 N NaOH + 19.16 cc. H <sub>2</sub> O	12.5
13	$0.6 \text{ cc. } 1  N \text{ H}_{9} PO_{4} + 0.26 \text{ cc. } 1  N \text{ a} O H + 19.14 \text{ cc. } H_{2} O \dots $	12.5
14	$0.6 \text{ cc. } 1  N \text{ H}_{9} PO_{4} + 0.28 \text{ cc. } 1  N \text{ aOH } + 19.12 \text{ cc. } \text{ H}_{2} O \dots $	12.5
15	0.6 cc. 1 N H <sub>3</sub> PO <sub>4</sub> +0.30 cc. 1 N NaOH +19.10 cc. H <sub>3</sub> O	12.5
16	0.6 cc. 1 N H <sub>1</sub> PO <sub>4</sub> +0.32 cc. 1 N NaOH +19.08 cc. H <sub>2</sub> O	12.5
17	0.6 cc. 1 N H <sub>4</sub> PO <sub>4</sub> +0.34 cc. 1 N NaOH +19.06 cc. H <sub>2</sub> O	12.5
18	0.6 cc. 1 N H <sub>3</sub> PO <sub>4</sub> +0.36 cc. 1 N NaOH +19.04 cc. H <sub>2</sub> O	12.5
19	0.6 cc. 1 N H <sub>4</sub> PO <sub>4</sub> +0.38 cc. 1 N NaOH +19.02 cc. H <sub>2</sub> O	12.5
20	0.6 cc. 1 N H <sub>3</sub> PO <sub>4</sub> +0.40 cc. 1 N NaOH +19.00 cc. H <sub>2</sub> O	13.0
21	0.6 cc. 1 N H <sub>3</sub> PO <sub>4</sub> +0.42 cc. 1 N NaOH +18.98 cc. H <sub>2</sub> O	14.5
22	0.6 cc. 1 N H <sub>3</sub> PO <sub>4</sub> +0.44 cc. 1 N NaOH +18.96 cc. H <sub>2</sub> O	15.0
23	0.6 cc. 1 N H <sub>3</sub> PO <sub>4</sub> +0.46 cc. 1 N NaOH +18.94 cc. H <sub>2</sub> O	16.0
24	0.6 cc. 1 N H <sub>3</sub> PO <sub>4</sub> +0.48 cc. 1 N NaOH +18.92 cc. H <sub>2</sub> O	17.0
25	0.6 cc. 1 N H <sub>3</sub> PO <sub>4</sub> + 0.50 cc. 1 N NaOH + 18.90 cc. H <sub>2</sub> O	75.0(?)
26	0.6 cc. 1 N H <sub>2</sub> PO <sub>4</sub> +0.52 cc. 1 N NaOH +18.88 cc. H <sub>2</sub> O	17.5
27	0.6 cc. 1 N H <sub>3</sub> PO <sub>4</sub> +0.54 cc. 1 N NaOH +18.86 cc. H <sub>2</sub> O	17.5
28	0.6 cc. 1 N H <sub>1</sub> PO <sub>4</sub> +0.56 cc. 1 N NaOH +18.84 cc. H <sub>2</sub> O	18.0
29	0.6 cc. 1 N H <sub>1</sub> PO <sub>4</sub> +0.58 cc. 1 N NaOH +18.82 cc. H <sub>2</sub> O	20.0
30	0.6 cc. 1 N H <sub>2</sub> PO <sub>4</sub> +0.60 cc. 1 N NaOH +18.80 cc. H <sub>2</sub> O	26.0
31	0.6 cc. 1 N H <sub>3</sub> PO <sub>4</sub> +0.62 cc. 1 N NaOH +18.78 cc. H <sub>2</sub> O	30.0
32	0.6 cc. I N H <sub>2</sub> PO <sub>4</sub> +0.64 cc. I N NaOH +18.76 cc. H <sub>2</sub> O	24.0
22	20 cc. water (control)	12.5

	Fibrin—Sodium Hydroxide + Increasing Amounts of Phosphoric Acid.	
Solution number.	H Concentration of solution.	eight of fibrin olumn in mm. lfter 24 hours
I	0.6 cc. 1 N NaOH +0.06 cc. 1 N H <sub>2</sub> PO <sub>4</sub> +18.80 cc. H <sub>2</sub> O	. 23.0
2	$0.6 \text{ cc. I } N \text{ NaOH } + 0.58 \text{ cc. I } N \text{ H}_3 \text{PO}_4 + 18.82 \text{ cc. H}_2 \text{O}_{$	. 23.5
3	0.6 cc. 1 N NaOH +0.54 cc. 1 N H <sub>2</sub> PO <sub>4</sub> +18.86 cc. H <sub>2</sub> O	. 25.5
4	0.6 cc. 1 N NaOH +0.50 cc. 1 N H <sub>a</sub> PO <sub>4</sub> +18.90 cc. H <sub>2</sub> O	. 28.0
5	0.6 cc. 1 N NaOH +0.46 cc. 1 N H <sub>3</sub> PO <sub>4</sub> +18.94 cc. H <sub>2</sub> O	. 32.5
6	0.6 cc. 1 N NaOH +0.42 cc. 1 N H <sub>3</sub> PO <sub>4</sub> +18.98 cc. H <sub>2</sub> O	. 35.5
7	0.6 cc. 1 N NaOH +0.38 cc. 1 N H <sub>3</sub> PO <sub>4</sub> +19.02 cc. H <sub>2</sub> O	. 40.5
8	0.6 cc. 1 N NaOH +0.34 cc. 1 N H <sub>3</sub> PO <sub>4</sub> +19.06 cc. H <sub>2</sub> O	. 47.0
9	0.6 cc. 1 N NaOH +0.30 cc. 1 N H <sub>3</sub> PO <sub>4</sub> +19.10 cc. H <sub>2</sub> O	. 45.0
10	0.6 cc. 1 N NaOH +0.26 cc. 1 N H <sub>2</sub> PO <sub>4</sub> +19.14 cc. H <sub>2</sub> O	· 47·5
II	0.6 cc. 1 N NaOH +0.22 cc. 1 N H <sub>3</sub> PO <sub>4</sub> +19.18 cc. H <sub>2</sub> O	. 52.0
12	0.6 cc. 1 N NaOH +0.18 cc. 1 N H <sub>a</sub> PO <sub>4</sub> +19.22 cc. H <sub>2</sub> O	. 59.0
13	0.6 cc. 1 N NaOH +0.14 cc. 1 N H <sub>3</sub> PO <sub>4</sub> +19.26 cc. H <sub>2</sub> O	. 62.0
14	0.6 cc. 1 N NaOH +0.10 cc. 1 N H <sub>3</sub> PO <sub>4</sub> +19.30 cc. H <sub>2</sub> O	. 63.5
15	0.6 cc. 1 N NaOH +0.06 cc. 1 N H <sub>3</sub> PO <sub>4</sub> +19.34 cc. H <sub>2</sub> O	. 68.0
16	0.6 cc. 1 N NaOH +0.02 cc. 1 N H <sub>3</sub> PO <sub>4</sub> +19.38 cc. H <sub>2</sub> O	. 75.0
17	20 cc. water (control)	. 12.5

TABLE VI.

#### III.

The experimental findings detailed in this note, with those previously published in the case of gelatin, bring out the fact that the minimal points of water absorption in citrate and phosphate mixtures are different in the cases of gelatin and fibrin. While the minimal swelling point for gelatin is found in a mixture closely approximating one of pure monosodium



citrate or pure monosodium phosphate, the minimal one for fibrin is found at a point represented more nearly by a mixture of molar equivalents of the monoand di- salts of these two acids. Such differences in the behavior of various proteins must be kept in mind when these experiments on simple protein colloids are applied to biological material. Protoplasm represents, according to our present notion, a mixture of at least two and probably several different proteins.

We hold that the results of this study, with those previously detailed upon the swelling of gelatin in polybasic acids and their salts, corroborate and amplify ideas previously expressed regarding the importance of acids, of alkalies, of various salts, and of these in mixture in determining the amount of water absorbed by protoplasm under physiological and pathological conditions. The well-established qualitative and quantitative analogies between the absorption of water by various hydrophilic colloids (like the proteins) and isolated cells,

organs or organisms, whether of animal or vegetable origin, show that the problem of water absorption is essentially a colloid-chemical phenomenon. These studies with polybasic acids and their salts permit us to re-emphasize the importance of an abnormal production or accumulation of acids within such colloid systems for increasing the 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).

Cincinnati, Ohio.

[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. Received November 12, 1917.

#### 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.<sup>1</sup> 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.

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