[Contribution from the Laboratory of Agricultural Chemistry of the University of Nebraska Experiment Station.]

ON THE COLLOIDAL SWELLING OF WHEAT GLUTEN.[1]

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The colloidal swelling of wheat gluten is of interest in connection with the general problem of water absorption by animal and plant tissues as elaborately investigated by Fischer[2] and his coworkers, and in connection with the theory and practice of bread making. Wood[3] and Wood and Hardy[4] have shown that wheat gluten is an emulsoid colloid and that when in contact with a solution its properties depend upon the concentration of acid and salts in that solution.

Wood's experiments were carried out by immersing small bits of gluten suspended over glass rods in beakers containing solutions of varying concentration of different acids, both with and without the presence of salts, and noting the effect of the different solutions on "disintegration" and "loss of cohesiveness" of the gluten. As these changes in the properties of the gluten are best regarded in our more modern terms of colloid chemistry as expressions of a change in the degree of dispersion of the colloid, and as these changes while often associated with, have in reality nothing to do with the changes in the *hydration capacity* of the colloid, it seemed to us that our experiments dealing with this second problem were worthy of record, expecially since the way in which they were carried out was an entirely different one from that adopted by Wood and Hardy. The experiments are also of interest in that they substantiate the important work of Fischer and others on the general problem of water absorption by proteins.

The colloidal swelling of animal proteins was first studied by F. Hofmeister.[5] It has since been extensively worked upon by Pauli,[6] Spiro,[7] Ostwald,[8] Fischer[2] and others. Gelatin swells more in the solution of any acid than it does in pure water. The amount of swelling in a given time for a given acid varies directly with the concentration of the acid

[1] This paper was originally accepted for publication in This Journal for July, 1914. The papers by Wood and by Wood and Hardy came to our notice after we had returned our proof. We withdrew our paper and now present it with the revisions made necessary because of the previous work of these two authors. The experimental details of our work were sent Dr. Fischer early in May, 1914, and are quoted by him in his "Oedema and Nephritis," second edition, New York, 1915, recently issued.

[2] Fischer, *Oedema and Nephritis*, Sec. Ed., N. Y. (1915).

[3] Wood, *J. Agr. Sci.*, **2**, 267 (1907).

[4] Wood and Hardy, *Proc. Roy. Soc. London*, (*B*) **81**, 38 (1909).

[5] Hofmeister, *Arch. Exp. Path. u. Pharm.*, **27**, 395 (1890).

[6] Pauli, *Pflüger's Arch.*, **67**, 219 (1897); **71**, 1 (1898).

[7] Spiro, *Beit. Chem. Physiol.*, **5**, 276 (1904).

[8] Ostwald, *Pflüger's Arch.*, **108**, 577 (1905).

up to a certain point. Thus Ostwald found that $N/38$ hydrochloric acid gave a maximum swelling of gelatin plates after twenty-four hours. With increasing concentrations of the acid beyond this point, the absorption of water became less.

While gelatin swells more in the solution of any acid than it does in pure water, the various acids do not bring about the same degree of swelling when equinormal solutions are compared. Ostwald found the order in which the different acids cause gelatin to swell to be: hydrochloric, nitric, acetic, sulfuric, boric.

The addition of any salt to the solution of an acid decreases the amount that gelatin will swell in that solution. Fischer[1] has shown that, for a given acid, the effect of the salt in decreasing the amount of swelling of gelatin increases with increasing concentration of the salt. If the concentration of salt is made high enough the effect of the acid may be almost or entirely suppressed.

Equimolar solutions of different salts do not produce the same effect in diminishing water absorption by gelatin. Thus Fischer found when different sodium salts are compared that the chloride and nitrate show the least effect, the phosphate and tartrate the greatest, the acetate and sulfate an intermediate one. Again when different salts of the same cation with different anions were compared (as a series of chlorides), he found that the ions like those of the monovalent alkalies show the least retarding effect, those of the trivalent metals like iron and aluminium the greatest, while the bivalent ones of calcium and magnesium fall between.

A further fact is to be noted. Nonelectrolytes, like sugar, alcohol and urea produce a much less marked antagonistic effect on the absorption of water by gelatin in acid and neutral solution than do neutral salts.[2]

Fischer finds that what holds for gelatin as regards water absorption holds also for fibrin[3] and indeed for animal colloids[4] in general, such as frog muscle, sheep eyes, catgut and the like.

Experimental Part.

The gluten for the following experiments was prepared by washing the starch from flour under a stream of distilled water. We found the character of the gluten to be quite different when tap-water, which contains salts, was used in place of distilled water, being tougher and more elastic and smaller in bulk, just as pointed out by Wood and Hardy.[5] Since we wished to avoid the effect of any salts which might be absorbed by the gluten from the tap-water, all samples of gluten were prepared

[1] *Loc. cit.*

[2] Fischer and Sykes, *Science*, **37**, 486 (1913); *Koll. Z.*, **14**, 215 (1914).

[3] Fischer and Moore, *Am. J. Physiol.*, **20**, 313 (1907); *Pflüger's Arch.*, **125**, 99 (1908); *Koll. Z.*, **5**, 197 (1909).

[4] Fischer, *Pflüger's Arch.*, **124**, 69 (1907).

[5] Wood and Hardy, *Proc. Roy. Soc. London*, (*B*) **81**, 38 (1909).

with distilled water. The gluten ball was pressed out between glass plates to a fairly uniform thickness. After standing some time between the plates (during which time some fluid was usually squeezed off), discs could be cut from the gluten with a large cork borer, which were fairly uniform as to surface and weight. The discs were weighed to the nearest centigram and placed in the solution for exactly two hours, when they were removed, drained on a Buchner funnel and weighed again. The method is necessarily somewhat crude because the gluten is moist when weighed originally and because of the variation in the amount of water which mechanically adheres to the discs. Nevertheless, when the average of a number of determinations is taken the results are surprisingly uniform.

TABLE I.—LACTIC ACID.

Conc. of acid.	Wt. of water absorbed in g. per g. of moist gluten.				Average.
	a.	*b.*	*c.*	*d.*	
None	0.046	...	0.075	0.043	0.055
0.002 *N*	1.30	1.31	1.02	1.07	1.18
0.005 *N*	1.42	1.54	1.35	1.55	1.46
0.01 *N*	1.51	1.77	1.44	1.55	1.57
0.02 *N*	1.60	1.55	1.53	1.61	1.57
0.04 *N*	1.48	1.51	1.35	1.42	1.44
0.1 *N*	1.37	1.38	1.07	1.28	1.27
0.2 *N*	1.23	1.15	1.11	1.19	1.15
0.5 *N*	1.01	Lost	0.99	1.08	1.03

TABLE II.—ACETIC ACID.

Conc. of acid.	Wt. of water absorbed in g. per g. of moist gluten.			Average.
	a.	*b.*	*c.*	
None	0.03	0.01	—0.03	0.01
0.002 *N*	1.11	1.47	1.30	1.29
0.005 *N*	1.39	1.58	1.90	1.62
0.01 *N*	1.43	1.58	1.87	1.63
0.02 *N*	1.56	1.76	1.96	1.76
0.04 *N*	1.80	1.88	2.03	1.90
0.1 *N*	1.62	2.06	1.86	1.85
0.2 *N*	1.51	1.82	1.76	1.69
0.5 *N*	1.49	1.69	1.66	1.61

TABLE III.—HYDROCHLORIC ACID.

Conc. of acid.	Wt. of water absorbed in g. per g. of moist gluten.		Average.
	a.	*b.*	
None	0.00	0.00	0.00
0.002 *N*	1.47	1.28	1.37
0.005 *N*	1.63	1.44	1.54
0.01 *N*	1.67	1.37	1.52
0.02 *N*	1.37	1.12	1.23
0.04 *N*	0.83	0.68	0.75
0.1 *N*	0.14	0.16	0.15
0.2 *N*	—0.097	0.01	—0.04
0.5 *N*	—0.19	—0.09	—0.14

The curves showing the amount of water absorption with increasing concentration of acid for Tables I, II, and III are shown in Fig. 1. The curves represent the average for four, three and two determinations, respectively, for lactic, acetic, and hydrochloric acids. The concentrations of acid are plotted along the horizontal, and the water absorption in grams per gram of moist gluten is plotted on the vertical axis. An inspection of these curves brings out some interesting facts. For hydrochloric acid the maximum absorption is obtained with a concentration of 0.005 *N*, while the concentration for maximum absorption with lactic acid lies between 0.01 *N* and 0.02 *N* and for acetic acid is 0.04 *N*. It is to be noted that for concentrations above the one for maximum absorption, the curves do not fall off at anything like the same rate for the three acids. The curve for hydrochloric falls much more rapidly than the curves for the other two. This agrees with the results as found by Fischer[1] for fibrin and by Ostwald for gelatin.[2] Fischer obtained maximum swelling of both fibrin and gelatin in approximately 0.025 *N* hydrochloric acid and diminished swelling for concentration above this.

Of special interest is the fact that both lactic and acetic acids show concentrations of optimal swelling for gluten. Such an optimal swelling of a protein in a "weak" acid, has never before been observed. It does not occur in gelatin, fibrin and the other animal colloids thus far studied. The swelling of gluten also diminishes much more rapidly with increasing concentration of hydrochloric acid beyond the optimal point, than does the swelling of gelatin or fibrin. It is also of interest that moist gluten *loses* water in the higher concentrations of hydrochloric acid 0.2 *N* and 0.5 *N*. We have established that gluten discs lose weight in these higher concentrations of acid because of loss of water and not because of "solution" of the gluten. Examination of the surrounding fluids in these higher concentrations of acid fails to reveal more than traces of dissolved protein, whereas in the lower concentrations where greatest swelling takes place considerably more protein is dissolved. When gluten swells in dilute acid the discs puff up and take on an appearance somewhat resembling cotton balls, finally becoming transparent, soft and gelatinous. In 0.2 *N* and 0.5 *N* hydrochloric acid the discs do not change in appearance or in physical properties except to become tougher and more elastic just as in salt solutions. Discs which have lost water in 0.5 *N* hydrochloric acid gain water and become soft and gelatinous when placed in more dilute acid. Those which have absorbed water to more than double their weight in the more dilute acid, lose it if placed in 0.5 *N* acid. The taking up and giving off of water is, in other words, largely reversible.

When any salt is added to an acid in which a gluten disc is swelling,

[1] Fischer, *Oedema and Nephritis*, Sec. Ed., N. Y. (1915), pp. 44, 48.

[2] *Loc. cit.*

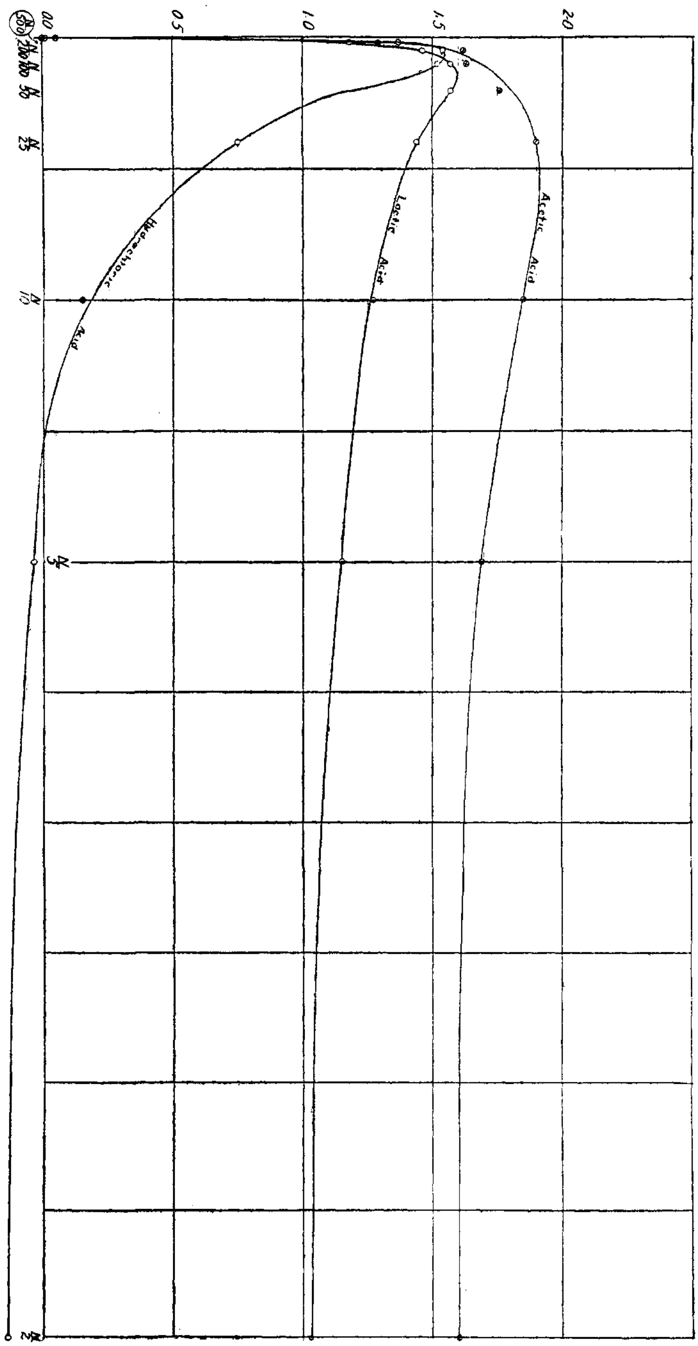
0.0
0.5
1.0
1.5
2.0
N/500
N/200
N/100
N/50
N/25
N/10
N/5
N/2
Hydrochloric Acid
Lactic Acid
Acetic Acid

Fig. 1.

the swelling is much reduced. This is shown in Table IV and Fig. 2 which is based upon the results shown in this table.

TABLE IV.—0.005 *M* SALT SOLUTIONS AND VARYING CONCENTRATIONS OF LACTIC ACID.

Conc. of acid solution.	Wt. of water absorbed in g. per g. of moist gluten. 0.005 *M* $K_2C_4H_4O_6$.	0.005 *M* K_2HPO_4.	0.005 *M* KCl.	0.005 *M*. $CaCl_2$.	No salt.
No acid	—0.11	—0.14	—0.11	—0.11	—0.01
0.002 *N*	—0.06	—0.012	0.49	0.25	1.39
0.005 *N*	0.04	0.16	0.77	0.42	1.50
0.01 *N*	0.25	0.63	0.98	0.57	1.81
0.02 *N*	0.55	0.86	1.28	0.68	1.87
0.04 *N*	0.73	0.97	1.30	0.89	1.72
0.1 *N*	1.06	1.12	1.43	0.96	1.69

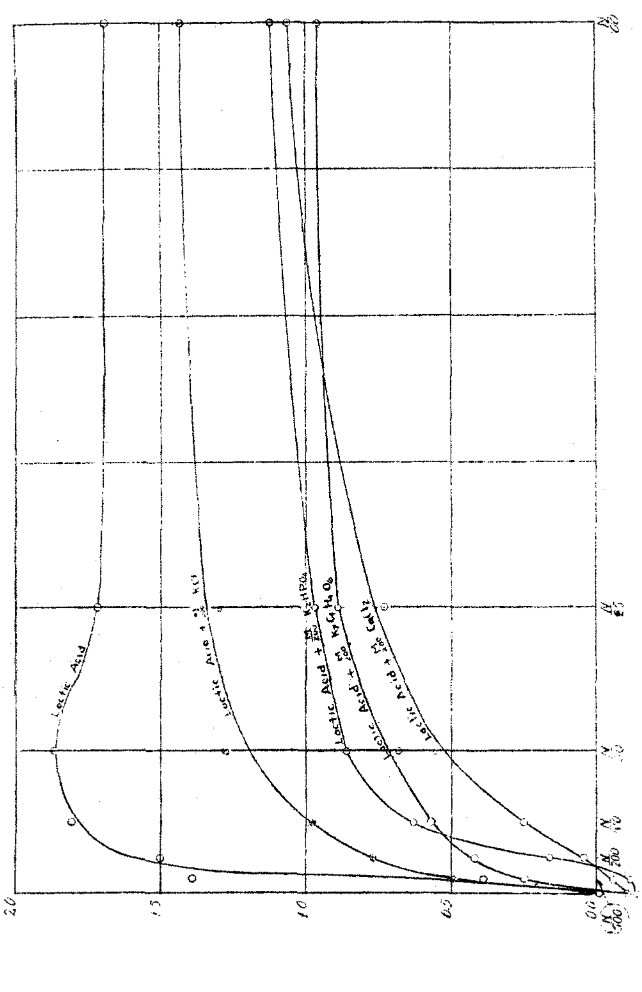

Fig. 2.

In Table V are given the figures showing the effect of varying concentrations of four salts and also of glycocoll on the absorption of water by gluten in the presence of 0.01 N lactic acid. The results are shown graphically in Fig. 3.

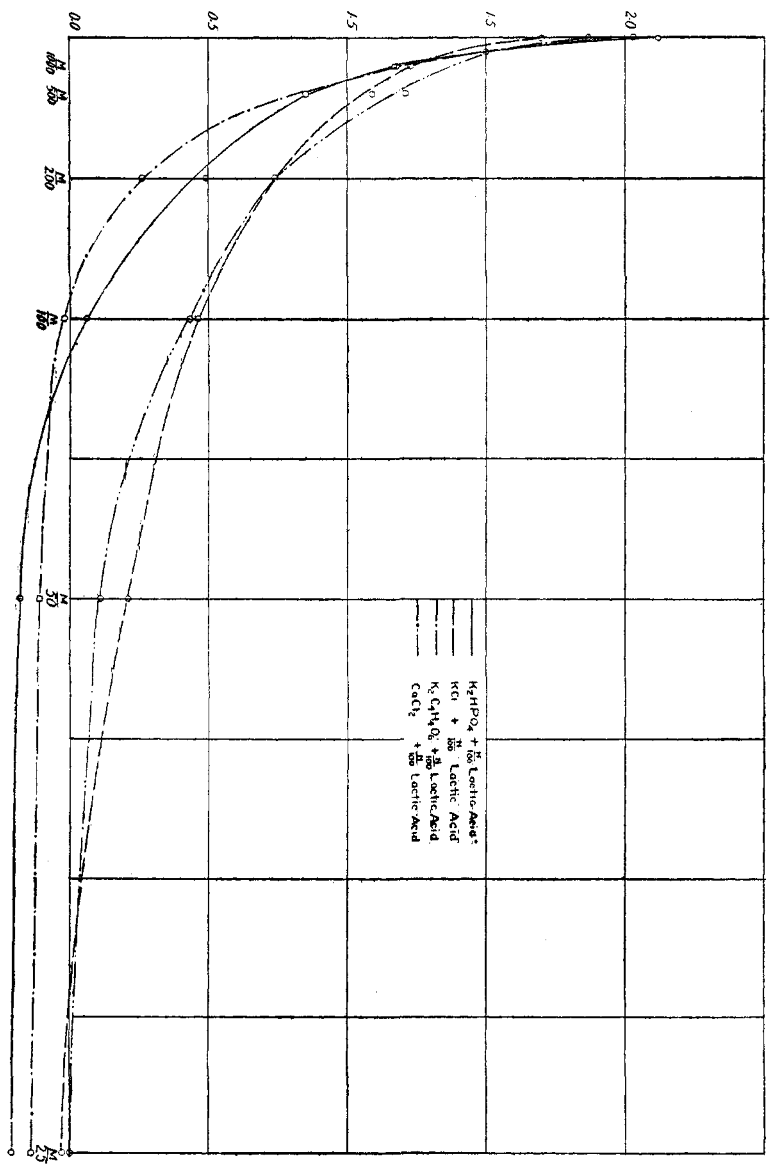

Fig. 3.

In all cases there is a rapid decrease in water absorption with increase in the concentration of the salt. In the higher concentrations of phosphate and tartrate the gluten disc drops to a weight below that of the original moist disc.

TABLE V.—0.01 *N* LACTIC ACID AND VARYING CONCENTRATIONS OF SALTS AND OF GLYCOCOLL.

Conc. of salt solution.	Wt. of water absorbed in g. per g. of moist gluten. 0.01 *N* lactic acid and				
	KCl.	K_2HPO_4.	$K_2C_4H_4O_6$.	$CaCl_2$.	Glycocoll.
No salt	1.70	2.12	1.87	2.02	1.70
0.0001 *M*	1.23	1.17	1.18	1.50	..
0.002 *M*	1.09	0.85	0.85	1.21	1.57
0.005 *M*	0.74	0.49	0.26	0.85	1.65
0.01 *M*	0.46	0.06	—0.02	0.43	1.29
0.02 *M*	0.21	—0.18	—0.11	0.11	1.21
0.04 *M*	—0.03	—0.21	—0.14	0.00	1.14
0.1 *M*	..	..	..	..	0.71
0.2 *M*	..	..	..	..	0.53
0.4 *M*	..	..	..	..	0.50

At the concentration of 0.02 *M* the order of salts as regards their effect in diminishing absorption is the same as found by Fischer for gelatin and fibrin, namely: chloride, tartrate, phosphate. That the relative position of the curves for the four salts is not the same for all concentrations may or may not be significant. We do not think the matter due to experimental error. There is undoubtedly a very important relation between the exact concentration of the acid and the inhibiting effect at different concentrations, of the salts. Of special interest is the fact that glycocoll behaves like a salt in reducing the swelling of gluten in acid solution. The effect, however, is less marked than with the different salts studied.

A series of photographs will help to make evident the significant differences in the swelling of gluten under different conditions.

Plate I.

Plate I shows the lactic acid series (*a*) of Table I. Beaker 1 contains distilled water. Beakers 2 to 7 contain lactic acid in concentrations from 0.002 *N* to 0.1 *N* as shown in Table I. The difference in size of the discs in distilled water and in the different concentrations of acid is plainly evident.

Plate II shows a 0.005 *N* lactic acid series, to which has been added varying concentrations of K_2HPO_4 as in Table V. Beaker 1 contains

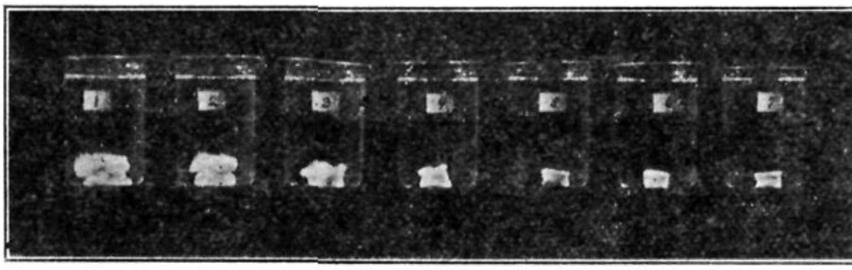

Plate II.

0.01 *N* lactic acid. Beakers 2 to 7 contain 0.01 *N* lactic acid with K_2HPO_4 varying in concentration from 0.01 *M* to 0.4 *M*. The increasing antagonistic effect of the salt with increasing concentration is very apparent.

TABLE VI.

A. Original wt. of discs.		B. Wt. of discs after 2 hrs. in acid.		Conc. of acid.	C. Wt. of discs after transference to 0.1 *N* K_2HPO_4 for 1 hr.	
a.	*b.*	*a.*	*b.*		*a.*	*b.*
1.39	1.23	1.41	1.20	No acid	1.14	0.95
1.30	1.26	3.21	2.89	0.002 *N*	1.18	1.16
1.33	1.22	3.43	3.54	0.005 *N*	1.22	1.10
1.39	1.28	3.57	3.67	0.01 *N*	1.27	1.17
1.42	1.24	3.92	3.67	0.02 *N*	1.30	1.14
1.29	1.27	3.90	3.85	0.04 *N*	1.17	1.15
1.29	1.29	3.95	3.69	0.1 *N*	1.21	1.24
1.31	1.29	3.69	3.56	0.2 *N N*	1.28	1.26
1.31	1.30	3.52	3.46	0.5 *N*	1.35	1.35

Table VI shows the reversible nature of water absorption by gluten. There are shown in Col. A the original weights of two series of gluten discs, and in Col. B their weights after remaining two hours in acetic acid solutions varying from 0.002 *N* to 0.5 *N*. In Col. C are given the weights of the same discs after remaining one hour in 0.1 *N* dipotassium phosphate solution. These experiments show how the discs, after taking up water in the acid solutions to twice their original weight or more, give up the water in the salt solution. Not only do they reassume their original weight, but their original appearance and physical properties as toughness and elasticity as well.

TABLE VII.—EFFECT OF TEMPERATURE ON WATER ABSORPTION.

Solution.	Wt. of water absorbed in g. per g. of moist gluten. 24°.	39°.
In water	0.06	0.09
In 0.01 *N* HCl	1.47	2.01
In 0.01 *N* lactic	1.65	2.66
In 0.01 *N* acetic	1.68	2.79

Table VII is introduced to show the effect of temperature on the absorption of water by gluten. It shows that gluten swells the more in acid solutions the higher the temperature.

TABLE VIII.—0.01 *N* LACTIC ACID WITH VARYING AMOUNTS OF FLOUR AND BRAN EXTRACTS.

0.01 *N* lactic acid cont. extract from flour.	Water abs. in g. per g. of moist gluten.	0.01 *N* lactic acid cont. extract from bran.	Water abs. in g. per g. of moist gluten.
No extract	2.23	No extract	1.48
0.625 g. per 100 cc.	2.16	0.625 g. per 100 cc.	1.12
1.25 g. per 100 cc.	1.81	1.25 g. per 100 cc.	1.02
2.50 g. per 100 cc.	1.47	1.87 g. per 100 cc.	0.91
5.00 g. per 100 cc.	1.25	2.50 g. per 100 cc.	0.82
		4.75 g. per 100 cc.	0.33

TABLE IX.—0.01 *N* LACTIC ACID WITH VARYING AMOUNTS F CANE SUGAR.

Conc. of sugar solution.	Water abs. in g. per g. of moist gluten.
None	1.75
0.1 *M*	1.41
0.2 *M*	1.54
0.5 *M*	1.42
1.0 *M*	0.71
1.5 *M*	0.28

Table VIII shows that water extracts of flour and bran reduce the swelling of gluten in acid solutions. Their effect is similar to, though not as marked, as the effect of neutral salts.

Table IX shows that nonelectrolytes such as cane sugar are comparatively ineffective in reducing the swelling of gluten in acid solutions except in high concentrations. Fischer finds the same for gelatin and sugar solutions.

The experiments described in this paper show that the mixture of vegetable proteins which comprises wheat gluten behaves in a manner entirely analogous to the animal colloids as studied by Fischer and others. Moist gluten absorbs water from acid solutions and the amount of absorption varies with the kind and concentration of the acid. The presence of neutral salts retards water absorption by gluten, and in the higher concentrations of salt may even cause loss of water from moist gluten. Gluten which has taken up water in an acid solution loses water and regains its original physical properties when placed in a salt solution. The nonelectrolytes are much less effective than electrolytes in inhibiting the swelling of gluten in acid solutions. These experiments therefore contribute to the important problem of the mechanism of water absorption and secretion by living plants which in animals has been proved by Fischer to be essentially a colloid phenomenon.

The facts brought out in our experiments, coupled with those of Wood and Hardy, are also of importance in relation to certain theoretical and practical problems of milling and baking. This topic we propose to discuss in a forthcoming bulletin from the Nebraska Experiment Station.

We would here express to Dr. Martin H. Fischer of the University of Cincinnati our appreciation of his interest in this work.

LINCOLN, NEBR.