THE ISOELECTRIC POINTS OF THE PROTEINS IN CERTAIN VEGETABLE JUICES.

By EDWIN J. COHN,* JOSEPH GROSS, and OMER C. JOHNSON.

(From the Harriman Research Laboratory, Roosevelt Hospital, New York, in Collaboration with the Section of Food and Nutrition, Medical Department, U. S. Army.)

(Received for publication, August 21, 1919.)

The preparation in the dry condition of systems rich in protein has assumed new importance in the practise of dehydrating vegetables and meats. Until recently the removal of water and the preservation of the original appearance of the foodstuffs have been considered the most important criteria for success in the operation. But acceptability of the dehydrated product has varied largely. In certain instances vegetables have lost important nutritional qualities upon dehydration or shortly thereafter. Others have been slow to reabsorb water. Occasionally dehydrated material has spoiled.

Information regarding the nature of the proteins in such systems should explain, in part, the causes of variation, set up criteria for change upon dehydration, and lead gradually to the perfection of processes.

With this in view the characteristics of the proteins in the juices of the potato, of the carrot, and of the tomato have been studied. Their isoelectric points and their solubility at different hydrogen ion concentrations have been determined. The acidity of the juices of these vegetables together with this information suggests the state in which the proteins exist in nature.

The Significance of the Isoelectric Point.

The classification of simple proteins depends upon their solubility in water containing different concentrations of inorganic salts (albumins from globulins), different concentrations of hydrogen ions (albumins and globulins from glutelins), or different concentrations of alcohol

* Lieutenant, Sanitary Corps, U. S. Army.

(prolamines).[1] But within each group state is affected by change in concentration of these factors. Thus the solubility of an albumin, a globulin, or a glutelin is variable with the amount of alcohol contained in the solvent; and the solubility of an albumin or a globulin is variable with the hydrogen ion concentration, and with the nature and amount of the dissolved electrolytes.

Recent investigations have suggested the course of certain of the chemical reactions that involve changes in the solubility of proteins. It appears that, whatever the superimposed complexity resulting from the colloidal nature of the systems, the behavior of protein substances, whether simple or conjugated, is dependent in large part upon their ionization as amphoteric electrolytes.[2,3,4] As amphoteric electrolytes, proteins combine with either acids or bases, but at a particular hydrogen ion concentration they exist most nearly uncombined. The value of this singular point in ionization and in behavior is characteristic of each protein and is termed its isoelectric point. The isoelectric point is thus a measure of the relative strength of protein as acid and base.

The combination of the protein molecule with acid or with basic radicles effects a change in its solubility and in its hydration. The compounds with simple acids or bases vary greatly in their ability to absorb water and to dissolve in water, but in the neighborhood of the isoelectric point protein substances are usually less soluble and less swollen than elsewhere. Empirical evidence for this conclusion antedates the theory for this phenomenon. Both in the laboratory and in industry the preparation of proteins has depended largely upon their lesser solubility near the isoelectric point.

The Determination of the Isoelectric Point.

The migration of charged particles in an electric field is termed cataphoresis. The charge of a protein depends upon its ionization.

[1] Recommendations of the Committee on Protein Nomenclature, *Am. J. Physiol.*, 1908, xxi, p. xxvii.

[2] Hardy, W. B., *Proc. Roy. Soc. London, Series B*, 1907, lxxix, 413. Sörensen, S. P. L., *Compt. rend. trav. Lab. Carlsberg*, 1917, xii.

[3] Loeb, J., *J. Gen. Physiol.*, 1918–19, i, 237.

[4] Henderson, L. J., Cohn, E. J., Cathcart, P. H., Wachman, J. D., and Fenn, W. O., *J. Gen. Physiol.*, 1918–19, i, 459.

Proteins can ionize as acids or as bases. As acids they migrate to the anode, as bases to the cathode. The nature of the ionization of the protein can thus be inferred from the direction of its migration. When it does not migrate in either direction the protein exists at its isoelectric point.

The isoelectric point of certain vegetable proteins was determined by the method of cataphoresis. The juice of the vegetable was placed in a U-tube, between electrodes charged with a potential difference, and the direction of the protein migration determined. The migration of protein during cataphoresis was followed both by determining nitrogen in the arms of the U-tube and by heating the liquid from the arms and noting in it the appearance of coagulated protein. The technique described by Coehn[5] and by Michaelis[6] was so modified as to meet the needs of the present research.

Unless several precautions are observed in investigating the direction and amount of the migration of protein compounds in an electric field the results are scarcely interpretable. The dipping of electrodes into the protein solution especially complicates the phenomenon[7] since proteins are either precipitated or rendered more soluble at the electrodes.[8] This is brought about, in part, by the accumulation of H^+ and OH^- ions at the cathode and anode respectively. Landsteiner and Pauli obviated these disturbances by using a three chambered vessel in determining the isoelectric point of very pure egg albumin.[9] Each chamber contained the protein solution, and migration was detected by determining nitrogen in each after the passage of current. Instead of protein solutions in the end-chambers Michaelis substituted a buffer solution of the same hydrogen ion concentration.

The particular form of apparatus devised for use in these experiments is represented in Fig. 1.[10] The protein solution was placed below and between the stop-cocks, BB, of a U-tube and above was

[5] Coehn, A., *Z. Electrochem.*, 1909, xv, 652.

[6] Michaelis, L., *Biochem. Z.*, 1909, xvi, 81.

[7] Haas, A. R. C., *J. Phys. Chem.*, 1918, xxii, 520.

[8] Robertson, T. B., *J. Phys. Chem.*, 1911, xv, 179.

[9] Landsteiner, K., and Pauli, W., *Verhandl. Kong. inn. Med.*, 1908, xxv, 571.

[10] The dimensions of the apparatus are of importance only in calculating movement of protein from the nitrogen concentration before and after cataphoresis. The volume of liquid contained in each arm was 7.4 cc. and in the central chamber below the arms (including stop-cocks) 13.4 cc.

placed a buffer solution of the same hydrogen ion concentration extending to stop-cocks, AA, and conveniently introduced at DD by creating a slight vacuum at C. Between the buffer solution (at A) and the zinc sulfate in the non-polarizable electrodes a sodium chloride solution was placed to avoid the formation of insoluble zinc salts. It was ascertained that the sodium chloride never met the protein by testing for the faster moving chlorine ion in a drop of solution pipetted from the arms of the U-tube. Once filled the level of the solutions was adjusted through C. The stop-cocks BB were then opened and, provided there was no diffusion, a 110 volt direct

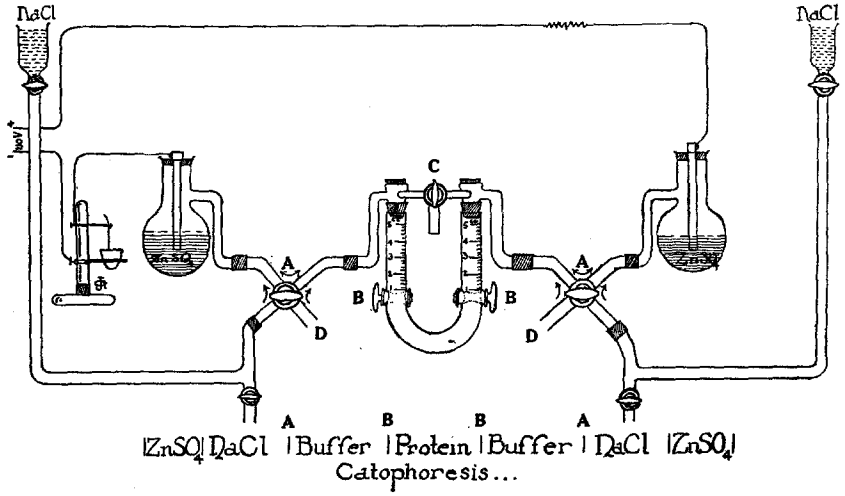

FIG. 1. Apparatus for the determination of protein migration in an electric field.

current was passed in series through the apparatus and a silver coulometer. The deposit of silver in the latter recorded the flow of current during cataphoresis. The drop in voltage across BB was approximately 3 volts, but varied in different experiments with the conductivity of the protein solution. Cataphoresis was carried on in a thermostat at 25° ± 1°C.

The Protein of the Potato.

About 8 per cent of the solids of the potato is protein and about 4 per cent is ash.[11] As a result, more than half the protein is dis-

[11] The average of all the values given in König, J., Chemie der menschlichen Nahrungs-und Genussmittel, Berlin, 4th edition, 1903, i, 713.

solved by the high concentration of electrolytes in the juice that can be squeezed from the potato. With the exception of a small amount of proteose only one well defined protein, the globulin tuberin,[12] has been isolated from the potato. It is present to the extent of from about 1 to 2 per cent in the juice, and was isolated and prepared from this and from the sodium chloride extract of the whole potato.

The Hydrogen Ion Concentration of Potato Juice.—The hydrogen ion concentration of the juice of the potato was slightly less than 10^{-6}N. When the juice was freed from suspended material—mostly starch—by filtration through pulp, the apparent hydrogen ion concentration was further reduced to about 10^{-7}N. It was then perfectly clear but darkened by the action of an oxidase intimately related to the globulin in location and in behavior. The oxidase was more active the lower the hydrogen ion concentration. A study of the action of the oxidase has been reported from this laboratory.[13]

The Precipitation of Tuberin —A precipitate separated upon the addition to the juice of the potato of either acid or alkali. The alkaline precipitate was less voluminous, more gelatinous, and more variable in amount than the acid precipitate. It was at first soluble in excess alkali, but later was denatured. Upon the addition of acid to potato juice a white, flocculent precipitate[14] appeared at about pH 6. The precipitate increased in amount and then gradually redissolved upon the addition of more acid. At acidities greater than those reported a protein precipitate gradually reappeared. This precipitate increased with increase in acidity and time and did not resemble a globulin in behavior.

The volumes of the precipitates that settled when different amounts of acid were added to potato juice in cylindrical vessels are recorded in Table I. In every instance the greatest precipitation appeared at a hydrogen ion concentration near 10^{-4}N. In bulk, however, and rate of settling, the precipitates varied to an extent which could not

[12] Osborne, T. B., and Campbell, G. F., *J. Am. Chem. Soc.*, 1896, xviii, 575.

[13] Falk, K. G., McGuire, G., and Blount, E., *J. Biol. Chem.*, 1919, xxxviii, 229.

[14] Upon standing, the protein frequently separated as a result of acid produced by bacteria.

be accounted for by differences in procedure. This variation was no greater than the variation in the amount of tuberin in the juice, but neither the amount of the precipitate nor its volume was dependent merely upon the concentration of protein. More probably it was related to differences in the content of electrolytes in the juice, for the addition of sodium chloride had a great effect upon the behavior both of the acid and of the alkaline precipitate.

TABLE I.

Measurements on Potato Juice.

N HCl added to 100 cc. of potato juice.	Gm. of nitrogen in potato juice.														
	0.213	0.238	0.242	0.292	0.309	0.310	0.213	0.238	0.286	0.315	0.213	0.238	0.242	0.309	0.310
	Volume of protein precipitate.						Nitrogen in filtrate from precipitate.				pH of filtrate from precipitate.				
cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*					
16		0						0.225							
15				0	0	1			0.275					2.18	2.30
13	9						0.210				2.90				
12						7									2.74
10	25			7	17		0.178		0.242		3.15			2.95	
8		12	80	22	54	20		0.182	0.243			2.75	3.45	3.35	3.57
7	84						0.179				3.60				
6			93	61	64				0.239	0.240			3.85	3.84	
5	88		96		68	23	0.187		0.251	0.242	4.27		4.27	4.12	4.32
4		30	91	30	82			0.200	0.251	0.248		4.07		4.39	
3	92				56	17	0.204		0.251	0.249	4.80			4.73	4.93
2		18			18			0.199		0.255		4.60		5.35	
1		4			0	0		0.220				5.45		6.31	6.15
0	0	0		0	0	0	0.213	0.238	0.286	0.315		6.38		7.00	6.80
N NaOH															
1		4			12	6				0.291		7.65		7.78	7.55
2		9			15	6				0.290		8.66		8.22	8.19
4		5			16	6				0.293		9.50		8.84	8.89
6		3			18	7				0.301				9.39	9.35
8					34									9.78	

This is illustrated by the experiment recorded in Table II in which different amounts of sodium chloride were added to juice to which the same amount of hydrochloric acid had been added. The rates of settling and the volumes of the resulting precipitates were observed

and the nitrogen content of the juices was determined after the precipitate had been removed by centrifuging. Small amounts of sodium chloride greatly decreased the volume of the precipitate and also increased the solubility of the tuberin, though not to the same extent. The effect of sodium chloride upon the solubility of tuberin and upon the apparent hydrogen potential of systems containing it is different not only in amount but in kind at different hydrogen ion concentrations. The relation between this change and the isoelectric point will be considered in another report.

TABLE II.

Effect of Sodium Chloride upon the Precipitation of Tuberin by Acid.

NaCl added to 100 cc. of potato juice + 5 cc. of N HCl.	Volume of precipitate.	Nitrogen in filtrate.
gm.	*cc.*	*gm.*
0	97	0.192
0.5	98	0.195
1.0	21	0.204
2.0	20	0.199
4.0	29	0.196
8.0	61	0.178

The Solubility of Tuberin.—The solubility of tuberin at different hydrogen ion concentrations was estimated by determining the nitrogen content of potato juice to which sodium hydroxide or hydrochloric acid had been added in different amounts and from which any resulting precipitate had been removed by filtering or centrifuging. The results of four different experiments, in two of which the volumes of the precipitate were also determined, are reported in Table I. In each tuberin was least soluble in the juice of the potato at hydrogen ion concentrations slightly greater than 10^{-4}N, where precipitation occurred. Precipitation also occurred at hydrogen ion concentrations near 10^{-8}N. The amount of these precipitates was very different, but neither the addition of acid nor of alkali completely precipitated the protein in potato juice. Indeed no more than 25 per cent of the total nitrogen in the juice of the potato was ever found in the most copious acid precipitate nor more than 8 per cent in the largest alkaline precipitate. A curve representing the solubility of tuberin in potato juice thus passes through two minima and a maximum, the latter at the reaction of potato juice (Fig. 2).

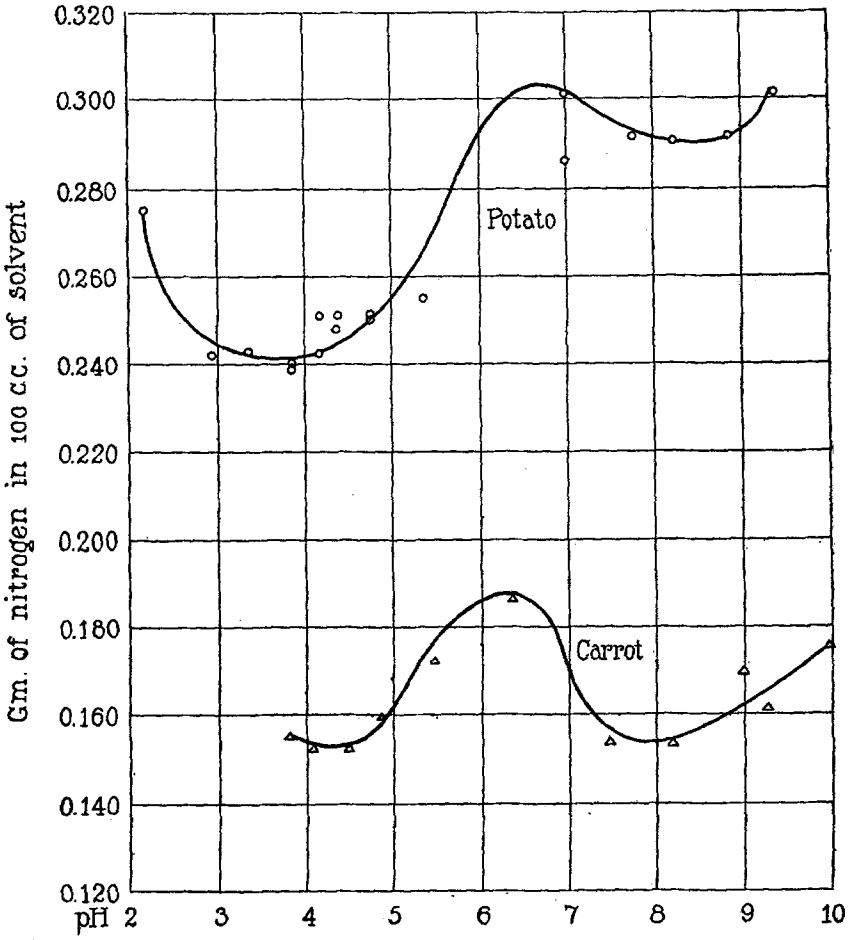

FIG. 2. Solubility of protein in vegetable juices at different hydrogen ion concentrations.

The Compounds of Tuberin.—The changes in the solubility of tuberin that have been described resulted, in part, from the combination with acids and bases of the basic and acid radicles in the juice of the potato. The amount of such combination is calculable if the hydrogen ion concentration is determined. It is equal to the difference between the measured hydrogen ion concentration and

that which would have resulted from the added acid or alkali had there been no combination. It is indicated by the changing slope of the curve representing the titration if the amount of normal acid or alkali is represented as ordinate and the hydrogen ion concentration as abscissæ (Fig. 3).

The curve representing the titration of potato juice with acid and alkali passes through a point of inflection at a hydrogen ion

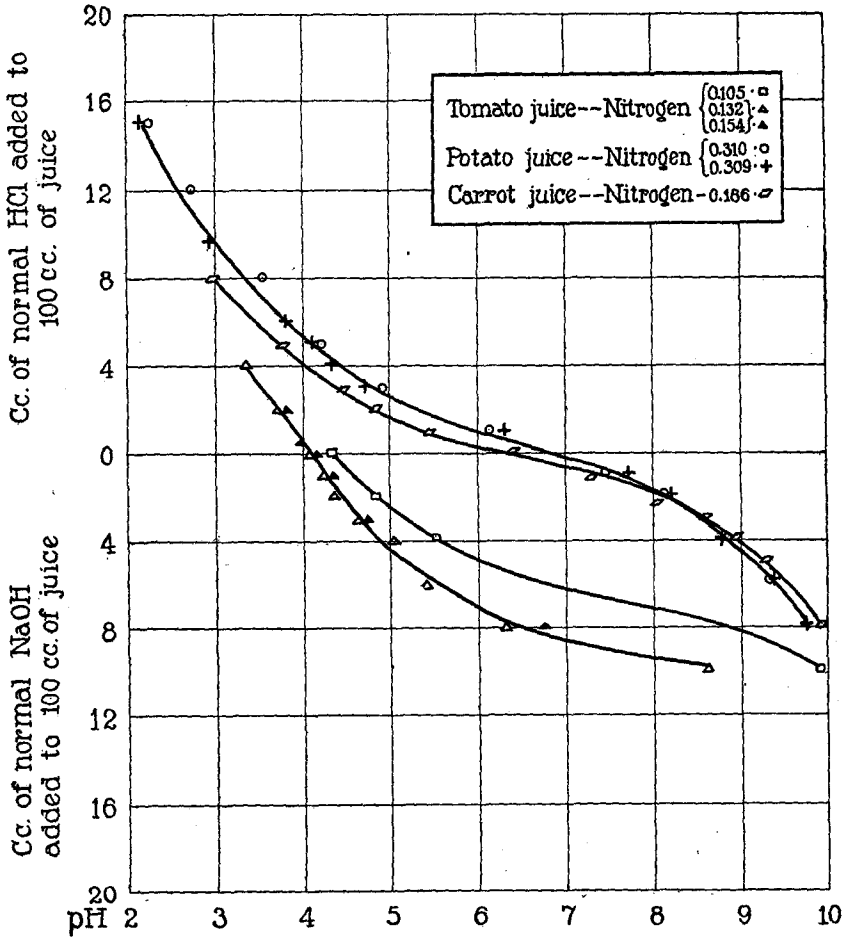

FIG. 3. Titration of vegetable juices.

concentration near the second ionization constant of phosphoric acid (1.6×10^{-7} = pH 6.8). But the strong buffer action, indicated by the steepness of the titration curve, throughout the range investigated cannot be attributed merely to the presence of phosphoric acid nor to the other organic and inorganic weak acids that chemical analyses reveal. The increase in steepness of the titration curve in the range, acid to pH 4.5 on the one hand and alkaline to pH 8.5 on the other, is largely due to the dissociation of the protein compounds that exist in the potato and their recombination with strong acids and bases with the retention of hydrogen and hydroxyl ions. The formation of new compounds at these particular reactions is suggested by a comparison of the titration curve (Fig. 3) with the curve representing the solubility of tuberin in the juice of the potato at different hydrogen ion concentrations (Fig. 2). Each change in solubility is seen to affect the slope of the titration curve, but in different ways. Thus, nearly three times as much acid was required to redissolve tuberin as to precipitate it. Osborne has made a similar observation on another globulin, edestin.[15]

The Isoelectric Point of Tuberin.—In an electric field the protein in the juice of the potato migrated toward the anode. It bore, therefore, a negative charge. No change followed an increase in the alkalinity of the juice. The direction of migration of the protein was, however, reversed by the addition of acid. In Table III are collected the results of many experiments. In all the direction of migration of the protein changed at a slightly lower hydrogen ion concentration than 10^{-4}N. At that acidity the protein did not migrate in either direction. It existed at its isoelectric point. At acidities greater than the isoelectric point protein migrated to the cathode. Under these circumstances tuberin apparently ionized as a base, and dissolved as an acid compound.

A second change in direction of the migration of protein in very acid juice occasionally occurred in our early experiments. This change was probably apparent and must be explained as relative to the increased passage of water at these acidities, since it was later averted by increasing the buffer in the arms of the cataphoresis apparatus.

[15] Osborne, T. B., *J. Am. Chem. Soc.*, 1902, xxiv, 39.

The flow of current during cataphoresis was measured, as has been said, by a silver coulometer in series with the apparatus. Since the juice of the potato contains both free electrolytes and electrolytes combined with tuberin, deductions regarding the transference of current by tuberin cannot readily be drawn from these data (Table III). It would be reasonable to suppose, however, that as the acidity of the solutions increased their specific conductivity would be increased

TABLE III.

Migration of Protein in Potato Juice during Cataphoresis.

N HCl added to 100 cc. of potato juice.	Amount of nitrogen in 5 cc. of filtrate.	pH of filtrate.	Conditions of cataphoresis.			Protein migration during cataphoresis.			
			Buffer solution used (M/20).		Flow of current.	Direction.	Amount of nitrogen after cataphoresis in 5 cc.		
			$C_2H_4O_2$	$C_2H_3O_2Na$	Silver per hr.		Anodal chamber.	Central chamber.	Cathodal chamber.
cc.	*mg.*		*cc.*	*cc.*	*gm.*		*mg.*	*mg.*	*mg.*
16			*			Cathodic.			
10	9.4	3.10	19.6	0.4	0.0091	"	(0.2	4.7	5.2)‡
8		3.45	19.0	1.0	0.0060	"			
7	8.4	3.60	18.5	1.5	0.0145	"	0.7	8.0	0.8
6		3.85	17.0	3.0	0.0185	"			
5	8.9	4.27	14.0	6.0	0.0338	"	0.5	7.2	0.6
5	11.1	4.27	14.0	6.0	0.0253	"			
4	12.1	4.50	12.0	8.0	0.0050	Anodic.	3.9	9.9	0.3
3		4.80	8.0	12.0	0.0048	"			
0	14.6	6.22	2.0†	8.0		"			

* Citrate mixture used.

† M/60 phosphate mixture used.

‡ Cataphoresis continued for 17½ hours.

because of the high mobility of hydrogen ions. None the less, the flow of current during cataphoresis seems to pass through a maximum at a hydrogen ion concentration slightly less than 10^{-4}N (Table III). Presumably conductivity passes through a maximum in the neighborhood of the isoelectric point.

The hydrogen ion concentration which numerically represents the isoelectric point of tuberin reveals the relatively stronger acid proper-

ties of this protein. The amount of acid required to titrate the protein to its isoelectric point is a measure of the amount of combined protein that exists in the juice of the potato and is dissociated by the addition of acid. The soluble salt of this protein that is formed on the other side of its isoelectric point evidently contains three times as much acid as was necessary to dissociate the compound that existed in nature. The relation between this compound and those that exist at other hydrogen ion concentrations and the state in which they exist are suggested by these data.

The Protein of the Carrot.

Remarkably similar in behavior to potato juice is the juice of the carrot. Existing at approximately the same hydrogen ion concentration it combines acid and alkali to precisely the same extent. Fig. 3 illustrates the essential coincidence of their titration curves. Moreover, protein separates from the juice of the carrot upon the addition either of acid or of alkali. The volume of the precipitate and the nitrogen in the filtrate are both recorded in Table IV. As in the potato, the acid precipitate of greatest bulk appeared at a hydrogen ion concentration slightly less than 10^{-4}N. The alkaline precipitate appeared near 10^{-8}N.

The isoelectric point of the protein in juice filtered from the acid precipitate was determined. The change in direction of protein migration also occurred at a hydrogen ion concentration not far from 10^{-4}N. All the measurements upon carrot juice are arranged in tabular form. With one exception—derived from the data upon its solubility—the salient characteristics of carrot protein suggest those of tuberin. The isoelectric point and amphoteric constants of the protein in both vegetables are essentially identical. In nature they exist at approximately the same reaction. A consideration of the curves representing their solubility at different hydrogen ion concentrations suggests that they may exist as somewhat similar compounds (Fig. 2). But whereas in the potato the alkaline precipitate is slight, in the carrot it is almost as great in amount as the acid precipitate.

TABLE IV.

Measurements on Carrot Juice.

N HCl added to 100 cc. of carrot juice.	Gm. of nitrogen in carrot juice.						Direction of protein migration.
	0.186	0.261	0.186	0.186	0.261	0.276	
	Volume of protein precipitate.		Nitrogen in filtrate from precipitate.	pH of filtrate from precipitate.			
cc.	*cc.*	*cc.*	*gm.*				
20		27			1.65		
16		26			2.21	2.10	Cathodic.
12		28			3.07	2.57	
10							
8	13	30		2.98		3.22	Cathodic.
6							
5	15		0.155	3.80			
4	17	36	0.152	4.06	4.37	4.23	
3	19		0.152	4.48	4.72		Anodic.
2	8		0.159	4.86			
1	0		0.172	5.45			Anodic.
0	0	8	0.186	6.43		6.25	"
N NaOH							
1	2		0.153	7.45			
2	5		0.153	8.18			
3	6		0.159	8.67			
4	6		0.169	8.95			
5	8		0.161	9.26			
8	14		0.175	9.92			

The Protein of the Tomato.

The juice of the tomato is very different. A high concentration of organic acids give to the ripe tomato a hydrogen ion concentration of nearly 10^{-4}N. According to Albahary,[16] the principal acids are malic, phosphoric, and citric. According also to Albahary,[17] the concentration of "albumin" and of "nucleoprotein" decreases during the period of maturation of the tomato and the concentration of free acids increases. As a result of a study of the buffer process in the metabolism of plants, Miss Hempel also suggested that the

[16] Albahary, J.-M., *Compt. rend. Acad. Sc.*, 1908, cxlvi, 336.

[17] Albahary, F.-M., *Compt. rend. Acad. Sc.*, 1908, cxlvii, 146.

extremely high acidity of the ripened lemon might be brought about in some such way.[18]

The lack of homogeneity of tomato juice that has merely been squeezed through cheese cloth is manifest. It contains, however, nearly 1 per cent of protein. Filtering the juice removes a large amount of this protein and at the same time appears to decrease the hydrogen ion concentration of the filtrate to nearly 10^{-5}N. Whatever

TABLE V.

Measurements on Tomato Juice.

N HCl added to 100 cc. of tomato juice.	Gm. of nitrogen in tomato juice.								
	0.105	0.132	0.154	0.105	0.132	0.154	0.105	0.132	0.154
	pH of tomato juice.			Direction of protein migration.			Silver deposited during cataphoresis.		
cc.							*gm.*	*gm.*	*gm.*
4		3.38							
2		3.71	3.80					0.0100	0.0380
1			4.00			Cathodic.			0.0380
0.6						"			0.0160
0	4.35	4.13	4.15	Cathodic.	Cathodic.		0.0609	0.0481	0.0243
N NaOH									
1		4.24	4.32			Cathodic.			0.0481
2	4.85	4.39		Cathodic.			0.0467		
3		4.63	4.79			Anodic.			0.0204
4	5.54	5.05		Anodic.	Anodic.		0.0321	0.0260	
6		5.42							
8		6.35	6.76						
10	9.90	8.68							
20	11.95								

the explanation of the latter observation, the removal of protein by filtration is easily understood. Protein exists largely in suspension.

In entire conformity are the results of cataphoresis (Table V). The unfiltered juice usually migrates to the cathode. A slight reduction in acidity reverses the sign of the protein. The protein, therefore, exists near its isoelectric point and probably slightly on the acid

[18] Hempel, J., *Compt. rend. trav. Lab. Carlsberg*, 1917, xiii.

side of it. In more nearly neutral solution it is soluble and can be freed from other constituents of the tomato by filtering through pulp. It then migrates to the anode. The addition of acid again precipitates the protein at its isoelectric point.

SUMMARY.

The state in which a protein substance exists depends upon the nature of its combination with acids or bases and is changed by change in the protein compound.

The nature of the compound of a protein that exists at any hydrogen ion concentration can be ascertained if the isoelectric point of the protein is known.

Accordingly information regarding the isoelectric points of vegetable proteins is of importance for operations in which it may be desirable to change the state of protein substances, as in the dehydration of vegetables.

The Protein in Potato Juice.—The hydrogen ion concentration of the filtered juice of the potato is in the neighborhood of 10^{-7}N. Such juice contains the globulin tuberin to the extent of from 1 to 2 per cent.

The character of the compound of tuberin that exists in nature was suggested by its anodic migration in an electric field.

The addition of acid to potato juice dissociated this compound and liberated tuberin at its isoelectric point. The isoelectric point of tuberin coincided with a slightly lower hydrogen ion concentration than 10^{-4}N. At that reaction it existed most nearly uncombined.

The flow of current during cataphoresis was greatest in the neighborhood of the isoelectric point. This evidence supplements that of the direction of the migration of tuberin, since it also suggests the existence of the greatest number of uncombined ions near this point.

At acidities greater than the isoelectric point tuberin combined with acid. The compound that was formed contained nearly three times as much acid as was needed to dissociate the tuberin compound that existed in nature. At such acidities tuberin migrated to the cathode.

Though never completely precipitated tuberin was least soluble in the juice of the potato in the neighborhood of its isoelectric point.

Both the compounds of tuberin with acids and with bases were more soluble in the juice than was uncombined tuberin.

The nature of the slight precipitate that separated when potato juice was made slightly alkaline was not determined.

The Protein in Carrot Juice.—The isoelectric point of the protein in carrot juice coincided with that of tuberin. Remarkably similar also were the properties of carrot juice and the juice of the potato. Existing in nature at nearly the same reaction they combined with acids and bases to nearly the same extent and showed minima in solubility at the same hydrogen ion concentrations. The greatest difference in behavior concerned the alkaline precipitate which, in the carrot, was nearly as great as the acid precipitate.

The Protein in Tomato Juice.—The protein of the tomato existed in a precipitated form near its isoelectric point. Accordingly it was not present to any extent in filtered tomato juice. If, however, the considerable acidity at which the tomato exists was neutralized the protein dissolved and was filterable. It then migrated to the anode in an electric field. The addition of sufficient acid to make the hydrogen ion concentration slightly greater than 10^{-5}N again precipitated the protein at its isoelectric point. At greater acidities migration was cathodic.