XLIV. THE ISOELECTRIC POINTS OF SOME PLANT PROTEINS.

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Any attempt to define the living plant more precisely in terms of our present chemical or physical conceptions, is faced at the outset with the infinite possibilities which almost any given mass of living protoplasm apparently presents. The view, however, is generally accepted that many of the essential characters of living protoplasm are bound up with the peculiar properties of the proteins. While the protein properties in general are possibly better known than are those of the individual plant proteins, a number of isolated facts suggested to us that the typical plant protoplasm, although probably different chemically in different genera of plants, tended to show in nature a common reaction to the external medium and behaved as though possessing a negative electrical charge. The facts, moreover, appeared to be most readily explained on the assumption that the protoplasm was composed largely of proteins, and it followed as a natural result of Loeb's work [1922] that these proteins were in nature commonly on the alkaline side of their isoelectric points. The typical plant parenchymatous tissues have usually a sap reaction of about p_H 5·5–6·5. The isoelectric points of plant proteins appear to be almost entirely unknown. The following investigations were therefore undertaken to ascertain isoelectric points for a series of representative proteins.

Methods.

It is apparent from this brief introduction that our investigation must take the nature of a preliminary survey over a fairly wide field. We should have to include representatives of the typical groups of vegetable proteins, the globulins, albumins and prolamins, and since the majority of known vegetable proteins are reserve substances, it was obviously necessary to include also some which were undoubtedly physiologically active (*e.g.* leucosin and tuberin). The relatively simple plants, *Nitella* and yeast, were included because direct determinations on protoplasm (as distinct from extracted proteins) seemed possible in these cases. It is obvious that this widening of the field of enquiry must profoundly influence the methods adopted. The isoelectric point of an ampholyte is usually determined by cataphoresis [see

Burton, 1916] or by the precipitation method [well discussed by Cohn, 1922]. Cataphoresis is, however, not always possible, particularly in the case of the prolamins or of such proteins as glutenin and caseinogen, which are not soluble in water or salt solutions [Cohn, 1922] and this, in itself, suggested that the precipitation method would prove more generally useful in survey work such as ours. In addition, since plant proteins are not as a rule well known or well defined, it was anticipated that relatively crude products would often represent the only material available, and it was desired to consider the behaviour of crude extracts in order to consider the possibility of determinations of isoelectric points in cases where the proteins were unknown, or occurred in mixtures. (The bean globulins are taken in illustration of this point.) Under such circumstances the precipitation method and colorimetric determinations of hydrogen ion concentration appeared to be both more generally useful and more rapid, and refinement of technique seemed to be of subordinate importance. Our purpose is clearly better served by finding the approximate position of the isoelectric points of a number of proteins than by very accurate determinations of the isoelectric points of one or two. We do not wish to imply that either our results or precipitation methods are inaccurate. A number of other methods have been employed in these investigations which give concordant results, although reasons of space prevent their discussion here. Further, we examined tuberin and edestin and obtained the same results by our methods as other workers had previously obtained using cataphoresis and electrometric determinations of hydrogen ion concentration.

The details of the methods used are as follows. Ten or twenty cubic centimetres of a given protein "solution" were taken in each of a series of graduated tubes of uniform bore. The solutions were acidified or made alkaline to varying degrees and made up to uniform volume with distilled water, since in the case of salt extracts, the degree of precipitation would be affected by the degree of dilution of the original salt solution. They were then allowed to stand and at a fixed time after treatment, usually half-an-hour, were centrifuged on an electrically driven centrifuge (at 1500 revolutions per minute) for equal periods, usually of two minutes. The volumes of the precipitate were then measured, or if the precipitates were of markedly variable consistency, they were dried and weighed. Centrifuging served to eliminate the marked differences in the rate of sedimentation. It is probably essential in many cases, as on allowing the precipitates to settle slowly some may show marked changes in volume in periods of a few hours, probably owing to hydrolysis of the protein by acid or alkali. An example of this is discussed under the heading of bean globulins.

To bring the protein solutions to the required reactions, the following reagents were used:

(1) Hydrochloric acid or sodium hydroxide.
(2) Acetic acid or sodium hydroxide.
(3) Citric acid or disodium phosphate.

The last pair form buffer solutions of a sufficiently wide range for most biological purposes [McIlvaine, 1921]. It is important to use the same proportions of salt in all determinations and to vary the acid only, as the solubility of a protein is intimately connected with the salt content of the solution. Cohn [1922] has shown, however, that, if the salt content is constant, the apparent position of the isoelectric point is not significantly affected.

Although the results given are only those for one of the reagents mentioned above, the three sets have been used in most cases, the results being similar to those given. Only the methods stated were employed for leucosin, gliadin and *Nitella*.

In the majority of cases, where a single protein can be extracted in aqueous or salt solution, the precipitation method offers no great difficulty. We have employed both crude extracts and "purified" proteins—with the same results. In the crude extracts the maximum precipitation is usually less sharply marked however. When the tissue extract contains two or more proteins or their derivatives, difficulties may arise. Such a case is that of the bean globulins discussed below. A further difficulty is presented by leucosin (from wheat) which is not readily precipitated by acid or alkali. A modified method has been used in this instance.

In view of the large number of estimations required in a given series of observations, the colorimetric method of determining hydrogen ion concentration was employed with the usual precautions [Clark, 1920]. This involves the possibility of a "protein" error. Since the solutions employed were normally quite clear, and since a limited number of check readings with the hydrogen electrode gave concordant results, the error at or near the isoelectric point of the protein is probably not important. The "salt" error in colorimetric determinations does not affect the results since most of our estimations were made in the presence of minimal and similar quantities of salts. It may be noted that the results in the presence of the phosphate buffer solutions were not significantly different from those obtained by other methods.

Results.

1. *Broad Bean* (Vicia faba).

As in most leguminous seeds, the two chief proteins (globulins) in the cotyledons of broad bean are vicilin and legumin. These storage proteins are readily extracted from ground seeds in 10 % sodium chloride solution. The extract is filtered off and saturated with ammonium sulphate crystals, the globulins being then precipitated. They are washed and redissolved in dilute ammonium sulphate (1/100 saturation). Saturated ammonium sulphate solution is then added until the whole solution becomes 6/10 saturated in respect of this salt, when legumin is precipitated. It is purified by repeating the process and finally separating by dialysis. The filtrate from legumin is saturated with ammonium sulphate—when vicilin separates. This is purified

by repeating the whole process of solution, 6/10 and complete saturation and is then separated by dialysis. Vicilin in 1 % sodium chloride or dilute ammonium sulphate shows maximum precipitation, on acidifying, at p_H 3·4 or a little higher. Legumin in similar solutions shows maximum precipitation at p_H 4·6. These results are illustrated in the following table:

Table I.

Vicilin In 6/10 saturated ammonium sulphate solution			Legumin In 1 % sodium chloride solution		
Acid added cc. of *N* HCl	Precipitate cc.	p_H	Acid added cc. of *N* HCl	Precipitate cc.	p_H
0·1	1·4	5·0	0	0	5·2
0·2	1·7	4·4	0·1	0·5	4·8
0·3	2·3	3·8	0·2	1·0	4·7
0·4	2·7	3·4	0·3	1·3	4·6
0·6	1·7	3·2	0·4	1·15	4·4
0·8	1·6	3·0	0·6	1·0	4·0
1·0	1·3	2·9	0·8	0·9	3·6
1·2	1·1	2·8	1·0	0·8	3·2
0	·0	5·3	1·2	0·6	2·8

The same results were obtained using suspensions of the proteins in water. In this and subsequent tables where no values are given for the higher p_H values, little or no precipitation occurs up to p_H 9·0.

Crude extracts of bean globulins. In view of the possibility of having to examine unidentified proteins in relatively crude extracts, the behaviour of the bean globulins in crude extracts with 10 % sodium chloride solution was considered. The fresh extracts of ground seeds were filtered to remove starch and other suspended matter, and then made acid or alkaline in the usual way. If the solutions were centrifuged immediately (within five minutes) after changing the reaction, maximum precipitation occurred at p_H values of 4·6 and 3·4. The precipitation curve apparently shows the effects of both globulins, vicilin and legumin, when this method of procedure is adopted (Table II, I).

If the solutions are allowed to stand for 30 minutes or more after changing the reaction, maximum precipitation takes place at much higher hydrogen ion concentrations, particularly about p_H values of 3·3 and 2·0, and the point in the precipitation curve at p_H 4·6 almost disappears (Table II, II).

A somewhat similar result is obtained on allowing the precipitate obtained by centrifuging immediately after acidifying to stand for some time (6 to 24 hours). It is then found that the volume of the precipitate at p_H 4·6 tends to decrease, while much further precipitation occurs in the solutions of higher hydrogen ion concentration. It seems possible that hydrolysis of the legumin takes place on standing and that the decomposition products have isoelectric points at lower p_H values. Vicilin, however, appears to be less affected by this treatment and may possibly be derived from legumin. In dealing with crude extracts it seems advisable to centrifuge and measure the precipitate

immediately on changing the reaction, to avoid the possibility of hydrolysis of less stable proteins.

The proteins of the crude extracts may be readily separated by employing the fact that their isoelectric points occur in solutions of different p_H value. The crude extract is poured into excess of water acidified to give the required final p_H value. The precipitate can be filtered off, redissolved or suspended and its point of maximum precipitation determined. The figures obtained for legumin in this way show a rather lower p_H value (4·4) for the isoelectric point, while vicilin gives the same result, as when using the purified proteins (Table II, III, IV).

Table II. *Extracts of globulins from bean cotyledons.*

I		II		III		IV	
p_H	Precipitate in cc.	p_H	Precipitate in cc.	p_H	Precipitate in cc.	p_H	Precipitate in cc.
5·8	0	7·4	tr.	8·0	0	6·5	0
5·0	3·0	6·2	tr.	7·0	tr.	5·2	0·4
4·6	**4·2**	5·4	tr.	5·0	0·5	4·7	1·2
4·4	2·3	4·9	0·8	4·2	0·9	4·6	1·5
4·2	3·2	4·6	2·2	3·8	1·3	**4·4**	**3·7**
4·0	3·7	4·5	2·0	3·6	2·8	3·6	0·7
3·8	4·0	4·4	2·6	**3·4**	**5·3**	3·4	0·7
3·6	4·3	4·2	4·0	3·2	1·2	—	—
3·4	**4·8**	**3·3**	**5·2**	3·0	0·8	—	—
2·2	3·1	2·6	3·0	2·8	0·4	—	—
1·6	2·8	2·4	5·5	—	—	—	—
—	—	**2·0**	**6·0**	—	—	—	—

20 cc. of filtered sodium chloride solution taken; hydrochloric acid and sodium hydroxide used to change reaction.

I. Centrifuged immediately after treatment.

II. Centrifuged 30 minutes after treatment.

III. Redissolved precipitate after precipitating in excess acidified water at p_H 3·6 (vicilin).

IV. Redissolved precipitate after precipitating at p_H 4·7 (legumin).

Proteins of growing beans. The possibility exists that the physiologically active proteins of living tissues bear little or no relation to the reserve proteins. It seemed of interest, therefore, to attempt to determine the isoelectric points of the proteins found in growing beans. Using both aqueous and salt extracts we have only been able to obtain very small quantities of precipitable protein in extracts of fresh tissues. While it appears to be safe to say that on acidifying these extracts most precipitation occurs *near* p_H values of 3·4 and 4·5, the volumes of the precipitates are so small that nothing more precise can be stated. We hope to consider further the question of dealing with the physiologically active proteins in subsequent papers.

2. *Pea* (Pisum sativum).

The globulins, vicilin and legumin, obtained from peas behave in the same way as those from broad beans. Both seeds contain rather small quantities of an albumin, legumelin. We obtained this most readily from pea flour, by dialysing an aqueous extract until the globulins were precipitated, adding

from time to time a few drops of sodium hydroxide to keep the solution neutral. The solution is then filtered and the filtrate acidified, and the samples heated simultaneously to 45–50°.

Table III. *Precipitation of legumelin from* 20 *cc. of aqueous solution.*

cc. of *N* HCl added	p_H	Precipitate in mg.	cc. of *N* HCl added	p_H	Precipitate in mg.
0	6·8	0	1·2	4·1	167
0·1	6·4	0	1·5	3·9	57
0·3	5·9	107	2·0	3·6	0
0·5	5·1	119	3·0	3·1	0
0·7	4·7	150	4·0	2·7	0
1·0	4·3	163			

Legumelin obtained from broad beans behaved in a similar way. Maximum precipitation occurred about p_H 4·1–4·3 and the isoelectric point appears to be close to p_H 4·2. The difficulty about legumelin is, however, the uncertainty as to whether the first product is free from the globulin legumin or not, since the latter is also slightly soluble in water. Dialysis cannot be continued very long owing to the ease with which hydrolysis takes place.

3. *Wheat* (Triticum vulgare).

This was taken as being typical of the cereals. In wheat the two principal reserve proteins are glutenin and gliadin, both being present to the extent of about 4 %.

Glutenin was prepared by extracting ground wheat seeds several times with warm alcohol (90 %) to remove the gliadin, and then extracting with 0·1 % potassium hydroxide. The glutenin may be precipitated from this solution with acetic acid and purified by washing, redissolving and precipitating again. This standard method of preparation obviously depends on precipitation at the isoelectric point. We varied it by dissolving in 5 % acetic acid and precipitating with sodium hydroxide. Both solutions in acetic acid and in sodium hydroxide gave maximum precipitation at p_H 4·4–4·5. In the sodium hydroxide solution a broad zone of high precipitation occurs between p_H 4·1 and 5·0. This may be characteristic of glutenin or may be due to the presence of another amphoteric substance, for on dissolving glutenin in 0·2 % acetic acid (to obtain a solution about p_H 4·5) and then making alkaline, a slight precipitate about p_H 5·5 was obtained. It is doubtful whether this is protein. A minor precipitation maximum also occurs about p_H 3·6. The precipitate at this point is largely protein.

Typical results are embodied in Table IV.

Gliadin is present in the extract of wheat with 90 % alcohol. The solution after concentration by boiling, preferably under reduced pressure, is poured into water, when the gliadin is precipitated. It is purified by dissolving in 90 % alcohol and precipitating again.

Table IV. *Glutenin.* 20 *cc. of solutions taken.*

(*a*) 0·2 % NaOH solution			(*b*) 5 % acetic acid solution			(*c*) 0·2 % acetic acid solution		
Acid added cc. 5 % acetic	Precipitate cc.	p_H	Alkali added cc. 4 *N* NaOH	Precipitate cc.	p_H	Alkali added cc. *N*/10 NaOH	Precipitate cc.	p_H
0	0·2	10·2	0	0	3·2	0	0	4·6
0·6	1·0	8·0	0·2	**2·7**	**3·6**	2·2	0	4·7
1·0	2·8	6·9	0·4	1·2	3·8	2·6	0	4·8
1·4	3·3	5·0	0·6	1·6	4·0	3·0	0	—
2·0	3·5	4·8	0·8	2·6	4·2	3·4	tr.	4·9
3·2	3·7	4·6	1·0	2·0	4·3	4·0	0·2	5·0
3·6	3·8	4·5	1·2	**3·4**	**4·4**	4·6	**0·4**	**5·5**
4·0	**4·0**	**4·4**	1·4	**3·4**	**4·5**	5·4	0·2	5·8
4·4	3·7	4·3	1·6	1·8	4·6	6·0	tr.	6·2
5·0	3·7	4·2	1·8	1·6	4·8	6·8	0	9·6
6·0	3·0	4·0	2·0	1·2	5·0	—	—	—
8·0	2·4	3·4	2·2	—	6·2	—	—	—

McClendon [1917] gives the isoelectric point of gliadin as p_H 9·3 on the authority of Michaelis. We have no evidence in support of this. Gliadin is soluble in dilute alkalies or acids. On neutralising these solutions, gliadin is precipitated—but the precipitates appear to be of approximately equal volume over a rather wide range of p_H values (3·0–7·0). On pouring an alcoholic solution of gliadin into ten times the volume of water previously made acid or alkaline with HCl or KOH, a fine precipitate of the gliadin appears rapidly in solutions more acid than p_H 5·5, and the maximum turbidity lies between p_H 3·5 and p_H 5·5. The isoelectric point probably lies between these limits.

A series of equal volumes of solution of gliadin in 80 % alcohol was acidified or made alkaline with *N*/50 HCl or NaOH, each of the samples being made up to the same volume with distilled water, so that the volume of the alcoholic solution was four times that of the added acid and water. (The alcoholic solution thus became a 64 % alcohol solution.) Using this procedure, maximum precipitation was obtained about p_H 5·2, though the volume of protein precipitated was small. Though larger precipitates can be obtained by diluting further there is of course the danger of precipitating all the protein, which is insoluble in water.

Table V. *Precipitation of gliadin from* 40 *cc. of an alcoholic solution.*

cc. *N*/50 HCl added	cc. *N*/50 NaOH added	Precipitate cc.	p_H
1·2	—	0·15	3·6
0·8	—	0·20	3·9
0·4	—	0·20	4·2
0·2	—	0·35	4·4
0	0	0·40	4·6
—	1·0	0·50	5·0
—	2·0	**0·55**	**5·2**
—	3·0	0·45	5·5
—	4·0	tr.	5·7
—	5·0	—	6·4

The point of maximum precipitation thus appears to be about p_H 5·2.

On theoretical grounds it appears to be somewhat unlikely that gliadin has an isoelectric point of p_H 9·3. Its content of arginine is low, lysine is practically absent and the proportion of glutamic acid present is very high (about 40 %). An isoelectric point of p_H 9·3 would make it resemble lysine (9·0) and arginine (10·48) which stand almost alone even among the amino acids of protein degradations in this respect. In addition to the fact that the greater part of the amino acids obtained on hydrolysis of gliadin have isoelectric points in acid media, it is known that the dipeptides are usually more acid than the amino acids from which they are obtained. Thus the p_H values of the isoelectric points of glycine, alanine and leucine are respectively 6·6, 6·7, 6·5 while those of glycyl-glycine, alanyl-glycine and leucyl-glycine are 5·5, 5·5 and 5·7 respectively. This suggests that the peptide linking tends to increase dissociation of H· more than that of OH′.

Leucosin is the albumin present in wheat. There is less than 1 % in the endosperm, but about 5 % in the embryo [Osborne, 1909]. This protein is important as it is one of few vegetable proteins known which is physiologically active and not merely a storage form. It is best obtained in aqueous extracts of whole wheat meal, and is the only carefully studied vegetable protein which cannot easily be precipitated by acid. It is, however, coagulated on heating in fresh aqueous extract. The longer the extract stands the more acid it becomes and the more easily the protein is coagulated by heat [Osborne, 1909]. The only acid with which we could obtain a precipitate at room temperatures was nitric, and the precipitates were too small to be accurately measured. We therefore made use of the heat effect. Equal quantities of fresh aqueous extract were treated with varying proportions of acetic acid and then simultaneously heated to 47°. The precipitates varied in consistency and were therefore dried and weighed. Maximum precipitation occurred at p_H 4·5.

Table VI. *Precipitation of leucosin from* 20 *cc. of fresh extract.*

cc. *N*/10 acetic acid added	p_H	Precipitate in mg.	cc. *N*/10 acetic acid added	p_H	Precipitate in mg.
0	6·3	0	6·6	4·3	39
0·6	5·3	tr*	8·3	4·25	30
1·7	4·8	31	10	4·2	25
3·3	4·5	65	11·7	4·1	20
5·0	4·4	42			

* Solution becoming turbid.

4. *Potato* (Solanum tuberosum).

The principal protein extracted from potato is the globulin, tuberin, which can be obtained either in the expressed sap or in salt extractions. The isoelectric point of this protein was found to be at p_H 4·3–4·5 by Cohn, Gross, and Johnson [1919], a result we have confirmed—our estimations giving maximum precipitation at p_H 4·4–4·5. Using 10 % sodium chloride extractions of dried potato, the value was rather lower (p_H 4·2–4·4) and there was a secondary maximum of precipitation at p_H 3·2. Extraction of dried ground

potato with $N/100$ NaOH gave a solution from which the maximum precipitate was obtained at p_H 3·3, the precipitate giving protein reactions. There thus appears to be a second protein in potato, not obtained in quantity in the expressed sap, with an isoelectric point at p_H 3·2–3·3. It is possible that this may be a degradation product of tuberin since it is most apparent in tissue dried or treated with alkali.

Table VII. 10 % *NaCl extract of dry potato powder*—20 *cc. taken.*

cc. of N HCl added	Precipitated cc.	p_H	cc. of N HCl added	Precipitated cc.	p_H
0	0	5·4	0·8	1·1	3·7
0·2	0·8	4·6	1·0	1·0	3·4
0·3	1·4	4·4	1·2	1·8	3·2
0·4	1·8	4·3	1·4	1·2	2·7
0·6	1·5	3·9			

Yeast. Precipitation methods have already been utilised to determine the isoelectric points of small unicellular organisms [Eggarth and Bellows, 1922]. Yeast suspensions were found to precipitate most rapidly in the vicinity of p_H 3·0. The cells remained almost completely suspended in solutions of p_H 4·8–10·0. Maximum precipitation and the clearest supernatant liquids were obtained at p_H 3·3–3·5. Considerable precipitation occurs when the p_H value is lower than 4·6 and there may be a secondary substance present with an isoelectric point in this region (cf. the bean globulins). The main isoelectric point of yeast appears to lie near p_H 3·3.

Table VIII. *Yeast suspended in tap water.*

No.		Series I p_H	Series I Precipitate in cc.		Series II p_H	Series II Precipitate in cc.	
1	Water + NaOH	10·0	0·3	Very turbid	10	0·3	Very turbid
2		9·0	0·3		9·5	0·3	
3		8·0	0·3		9·0	0·3	
4		7·3	0·3		7·5	0·3	
5		6·3	0·3				
6	Water	4·8	0·4		4·8	0·4	Slightly turbid
7	Water + HCl	4·6	0·6	Turbid	4·4	0·7	
8		3·3	1·0	Clear	3·7	0·7	
9		2·9	0·8		3·5	0·8	Clear
10		2·4	0·7		3·3	0·9	
11		2·0	0·9		3·1	0·9	
12		1·4	0·85	Turbid	3·0	0·8	
					2·8	0·8	

8 and 9 precipitating very rapidly on adding acid.

Series II: sodium carbonate used for the alkaline solutions and acetic acid for the acid.

Nitella. This alga possesses long cells and a minimum of wall tissue, and it is easily obtained in quantity. On grinding up the tissues in water or in 10 % salt solution and filtering through cotton wool a slightly greenish suspension is obtained. If this is acidified the suspended matter slowly precipitates, the maximum precipitation occurring at p_H 4·6 after 24 hours. The precipitate gives protein reactions and microscopical examination shows it to consist chiefly of extruded masses of protoplasm—with a few embedded

chloroplasts. About one-tenth of the maximum precipitate consists of free starch grains. These apparently settle equally rapidly in all the solutions and are practically all precipitated within two hours. *Nitella* protoplasm has therefore an isoelectric point near p_H 4·7.

Table IX. *Precipitation of protein from* 20 *cc. aqueous extract of* Nitella.

cc. of $N/30$ HCl added	Precipitate in 2 hrs. (cc.)	Precipitate in 24 hrs. (cc.)	p_H
0	tr.	0·4	6·0
0·2	0·2	0·4	5·7
0·4	0·2	0·4	5·4
0·7	0·2	1·5	4·9
1·0	0·3	1·7	4·6
1·5	0·6	0·9	4·3
2·0	0·4	0·5	4·0

Edestin. The isoelectric point of edestin has been determined by Rona and Michaelis [1910], using the precipitation method and phosphate buffer solutions, to be at p_H 6·9. Later, using acetate buffers Michaelis and Mendelssohn [1914] obtained a value of p_H 5·6. Hitchcock [1922] estimated the isoelectric point of edestin to be between p_H 5·5 and 6·0 on the results of precipitation, osmotic pressure and cataphoresis observations. The samples of edestin used by Hitchcock combined with phosphoric acid in solutions of less than p_H 5·0. The estimations have been repeated using disodium phosphate-citric acid buffers and also using hydrochloric acid as the precipitating agent. For the specimens of edestin used (commercial and purified extracts from hemp seeds) maximum precipitation was obtained at lower p_H values than 5·6 (*e.g.* p_H 5·3, phosphate buffers) but the value obtained seems to depend on the previous treatment and purity of the product. No evidence has been obtained in confirmation of the value given by Rona and Michaelis of p_H 6·9.

Conclusion.

A representative series of plant proteins have thus been examined and the results indicate that the isoelectric points of plant proteins are usually about p_H 4·5 or less often about p_H 3·3. Edestin is the only exception to this though possibly gliadin may resemble edestin. The few additional records we have been able to find show the same features, the results being summarised in Table X.

Table X. *Summary of Isoelectric Points* (p_H *values*).

	p_H		p_H	
Vicilin	3·4	Edestin	5·6	Michaelis and Mendelssohn
Legumin	4·4–4·6		5·5–6·0	Hitchcock
Legumelin	4·2	Carrot globulin	4·1–4·4	Cohn, Gross and Johnson
Glutenin	4·4–4·5	Nucleoprotein (pancreas)	3·5	McClendon
Gliadin	3·5–5·5 (? 5·2)	Nucleoprotein (paratyphus bacilli)	4·0	—
Leucosin	4·5	Bacterium coli	3·2	Eggarth and Bellows
Tuberin	4·4	Bacillus of rabbit septicaemia	4·2	Northrop and Kruif [1922]
Potato	3·2	B. typhosum	3·5	—
Yeast	3·1–3·3	—	—	—
Nitella	4·6–4·7	—	—	—

We do not wish to discuss at present the theoretical questions suggested by this table, but only to draw attention to one feature of interest. Since the cell sap of parenchymatous tissue in plants is usually slightly acid (p_H 5·5–6·5) it is clear that the principal proteins are normally on the alkaline side of their isoelectric points and hence behave as anions. There are exceptions to this generalisation, notably in unripe fruits, and occasionally elsewhere. If, however, our results are as representative as we believe them to be, an important property of the protein constituents of plant protoplasm is indicated, which throws considerable light upon many questions of permeability and of metabolism.

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